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BIOLOGY FACULTY

DOCTORAL SCHOOL OF BIOLOGY

BEHAVIORAL AND BIOCHEMICAL ASPECTS IN THE STUDY OF IRRITABLE COLON SYNDROME - ANIMAL MODELS AND HUMAN PATIENTS

PHD THESIS SUMMARY

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IAŞI

2022

SPECIAL THANKS

Many thanks and regards to Mr. C.S. And Dr. Habil. Ciobîcă Alin - Stelian, the scientific leader of this doctoral thesis, who guided, supported and provided me with the favorable framework for the successful completion of the research.

I would also like to extend my warmest thanks to the members of the doctoral committee for their kindness in analyzing this paper.

In the same context, thanks are also given to the members of the steering committee, composed of Ms. Assoc. Dr. Zenovia Olteanu, Mr. Prof. Univ. Dr. Habil. Dumitru Cojocaru and Mrs. Head of Works. Dr. Sabina Cojocaru, who participated with suggestions, advice and guidance of a real help in the whole process of research and realization of this paper.

Thanks for a nice collaboration addressed to Ms. Dr. Ioana Miruna Balmuş, Mr. Dr. Radu Lefter, Ms. Dr. Ababei Daniela, along with thoughts of gratitude and friendship.

Last but not least, I would like to thank my family and friends for their unconditional support, for sharing both the joys and the difficulties that marked and crowned this stage of my life. My thanks go to every person who has given me help, advice, understanding, and encouragement without which I would not have been able to accomplish this work.

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LIST OF ABBREVIATIONS AND KEYWORDS

ACTH - adrenocorticotropic hormones;

ANCA - antineutrophil cytoplasmic antibody;

ANS - autonomic nervous system;

ASCA IgA - anti-Saccharomyces cerevisiae antibodies;

BDNF - neurotrophic factor derived from the brain;

CgA- chromogranrin A;

VOC - volatile organic compounds;

CRP-C-reactive protein;

EE - ethanolic extract;

ENS - enteric nervous system;

FC - fecal calprotectin;

GPX - glutathione peroxidase;

Human HBD-2 - β-defensin-2;

HPA - hypothalamic-pituitary-cortico-adrenal axis;

IBD - inflammatory bowel disease;

IBS - irritable bowel syndrome;

IBS-C - irritable bowel syndrome with constipation;

IBS-D - irritable bowel syndrome with diarrhea;

IBS-M - irritable bowel syndrome with mixed manifestations;

IEC - intestinal epithelial cells;

IELs - intraepithelial lymphocytes;

IL-1β - interleukin-1β;

LBT - respiratory lactose identification test;

MDA- malondialdehyde

ME - methanolic extract;

MF - multifactorial stress;

NMS - neonatal maternal separation;

NO - nitric oxide;

PD - Parkinson's disease;

PD-IBS - post-diverticulitis irritable bowel syndrome;

P-IBS - predominantly painful irritable bowel syndrome;

Pi-IBS - post-infectious irritable bowel syndrome;

PMN- polymorphonuclear granulocytes;

PSQI - Pittsburgh Sleep Quality Assessment Questionnaire

ROS - reactive oxygen species;

SC - restraint stress;

SCFA - short chain fatty acids (shortchainfattyacids);

SOD - superoxide dismutase;

TIMP-1- tissue inhibitor of metalloproteinase-1;

TNF- tumor necrosis factor;

U-IBS - unclassified irritable bowel syndrome;

VAS-IBS - visual analog scale for irritable bowel syndrome;

ESR - erythrocyte sedimentation rate;

XO - xanthine oxidase;

KEYWORDS

- Irritable bowel syndrome
- Gastrointestinal disorders
- ROME IV
- Biomarkers
- Gut-brain axis
- Oxidative stress
- Nitrosative stress
- Depression
- Anxiety
- Sleeping disorders
- VAS-IBS
- PSQI
- Inflammation
- Animal models
- Multifactorial stress
- Contention stress
- Maternal neonatal separation
- Y maze test
- Elevated plus maze test
- Forced swimming test
- Methanolic extract
- Ethanolic extract
- Polyphenolic extract
- *Camelina sativa* var. Madalina
- *Chrysanthellum americanum*
- Glutathione proxidase
- Superoxide dismutase
- Malondialdehyde
- Nitric oxide
- Xanthine oxidase
- Catalase
- Interleukins
- Serotonin
- Tumor necrosis factor
- Biomarkers from tears
- Urine biomarkers
- Biomarkers from faeces

INTRODUCTION

This paper brings to the fore an increasingly common pathology in clinical medical practice - irritable bowel syndrome (IBS). A considerable number of publications and specialized studies highlight the interest in the group of digestive functional disorders, in which irritable bowel syndrome is felt.

The pathogenesis of this condition, similar to other functional disorders, is not yet fully known. For this reason, multiple mechanisms have been proposed to explain the pathogenesis of this syndrome over time, but so far there is not a single mechanism leading to irritable bowel syndrome, thus accepting the prospect of an multifactorial etiopathogenesis.

Being defined by a variable pattern of symptoms, which can be present both in the small intestine and in the colon, the disorder is characterized by abdominal pain or discomfort, in which there are changes in intestinal transit. The most common symptoms present in irritable bowel syndrome are bloating, constipation, diarrhea, the feeling of incomplete defecation or the presence of mucus in the stool (Schmulson and Drossman, 2017).

Diagnosing irritable bowel syndrome is a complicated process, sometimes similar to a complex puzzle, that involves gathering and analyzing information. The ROME IV criteria, which support the diagnosis of irritable bowel syndrome, highlight the disruption of the functioning of the gastrointestinal tract and propose the concept of intestinal disorders closely correlated with the gut-brain axis. These conditions are characterized by digestive symptoms related to motility disorders, visceral hypersensitivity, being correlated with the processing of the stimulus of the microbiome, the immune system of the mucosa or the central nervous system (Gilkin Jr, 2005).

Currently, there is growing evidence of the role of the intestinal microbiome in the pathogenesis of this syndrome. Therefore, irritable bowel syndrome could be defined as a combination of chronic symptoms such as pain and disorders of intestinal transit (diarrhea or constipation), but resulting from altered intestinal microbiome, being closely correlated with changes in parietal immune response and increased sensitivity of the intestinal wall (Schmulson and Drossman, 2017). Following this paradigm shift, new therapeutic options must also be considered.

At the same time, the diagnosis must be accompanied by the exclusion of any metabolic or organic diseases, diabetes mellitus, thyroid disorders, lactose intolerance, malignant or benign conditions, psychiatric disorders or surgery with possible adhesions (Sullivan and Nord, 2005).

A well-known aspect is that the severity of the symptoms varies from one patient to another, with the predominance of one or more symptoms, the evolution of the disease being variable, unpredictable, often long-lasting, the remission stages alternating with the relapse phases of the symptoms. Due to the chronic nature of this condition, the cause of which has not yet been elucidated, in which emotional, dietary, medicinal or hormonal factors may promote or aggravate gastrointestinal symptoms, treatment is primarily symptomatic, with a focus on diet. Even though the pathology is known as "irritable bowel syndrome", imaging techniques cannot detect colon irritation.

As no specific IBS biomarker has been identified to date, attention has been drawn to the markers of oxidative stress, as recent studies have shown both signaling deficiencies and changes in oxidative balance in some gastrointestinal and neurological disorders. (Grenham et al., 2011; Mayer et al., 2015). In this regard, several studies have indicated the involvement of reactive oxygen species (ROS) in the development of IBS, with a significant decrease in antioxidant capacity not only in

patients. (Schwartz, Repine and Abraham, 1995; Mete *et al.*, 2013; Choghakhori *et al.*, 2017), but also in rat models with symptoms induced by irritable bowel syndrome. This was especially evident in the decrease in superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity in the intestinal tissue, and the increase in lipid peroxidation. (Mozaffari et al., 2011; Dolatabadi et al., 2018).

Given that no specific, targeted treatment to treat irritable bowel syndrome has been identified so far, the therapeutic technique should involve addressing the patient's problems, in particular prescribing treatments that act on the symptoms (Basnayake, 2018). A wide range of pharmaceutical treatments are currently used, most of which are designed for other indications, such as antispasmodics (Ruepert et al., 2011), peppermint oil (Camilleri and Boeckxstaens, 2017), tricyclic antidepressants and selective serotonin (Alexander C Ford et al., 2014), Rifaximin, an antibiotic that reduces the symptoms of IBS (Pimentel et al., 2011) or probiotics (Chey, 2017). Moreover, cognitive behavioral therapy, multi-component psychological therapy, dynamic psychotherapy, hypnotherapy are also used to relieve symptoms (Alexander C. Ford et al., 2014).

In the light of the above information regarding the current state of knowledge in this field, the fact that there are still a number of connections and elements of the etiology of the disease completely unknown, but also because so far there is no biomarker for diagnosing the disease. The elements proposed to be studied in this paper are motivating, especially in terms of understanding the complex mechanisms that this disease knows.

The main purpose of this paper is to evaluate and research different behavioral and biochemical aspects in human patients and animal models, aspects determined by different techniques, to establish possible correlations between the evaluated parameters and, last but not least, to provide characterization of a group of patients diagnosed with irritable bowel syndrome.

A first goal was to obtain some animal models of irritable bowel syndrome that replicated a number of mild symptoms of the condition. In this context, an attempt was made to highlight the effect of antioxidant treatments on irritable bowel syndrome in rats, based on the administration of an extract of *Chrysanthellum americanum L. Vatke*. Subsequently, behavioral issues and oxidative status of those animals were assessed.

Also related to experimental animal models, another objective was to compare some behavioral aspects and to evaluate the markers of oxidative stress in a mild experimental model of irritable bowel syndrome in mice subjected to the multifactorial stress paradigm. Subsequently, the antioxidant potential of the ethanolic and methanolic extract obtained from the seeds of *Camelina sativa* var. Mădălina was evaluated, both in terms of oxidative status and in terms of behavioral evaluation of animals subjected to the experiment.

Starting from these premises, a notable objective is that, from a neurological perspective, we want to know if the neuropsychiatric involvement in irritable bowel syndrome is relevant, moreover, if there is an overlap between the studied biochemical substrate and neuropsychiatric disorders.

Another objective of this paper is related to the assessment of emotional status in a group of patients diagnosed with IBS, both in terms of symptoms and in terms of how sleep and especially its dysfunctions affect their lives. In connection with the same group of patients, we evaluated the oxidative status from tear samples, taken by non-invasive methods, but also from serum, urine and feces.

The experiments presented in the paper were hosted by the Laboratory of Animal Physiology of the Faculty of Biology of the "Alexandru Ioan Cuza" University of Iasi and the Faculty of Veterinary Medicine, "Ion Ionescu de la Brad" University of Life Sciences, Iasi , St. Spiridon Hospital, Iasi, Oftaprof Medical Clinic, Iasi.

Studies based on animal experiments and those based on the participation of human patients and volunteers were carried out in compliance with European and national legislation governing these activities, and with the written and informed consent of the participants, the Ethics Commission of St. .Spiridon, Iași and the Ethics Commission of the Faculty of Biology of the "Alexandru Ioan Cuza" University of Iași, respectively the Ethics Commission of the Faculty of Veterinary Medicine, "Ion Ionescu de la Brad" University of Life Sciences, Iași.

CHAPTER 1. CURRENT STATE OF KNOWLEDGE IN THE FIELD

Irritable bowel syndrome is currently one of the most common functional gastrointestinal disorders. Being a functional dysfunction defined by a variable pattern of symptoms, which can occur in both the small intestine and large intestine, with a chronic progression whose diagnosis is often difficult to establish, this condition often requires multiple investigations and costs. A not inconsiderable aspect is the treatment, which is usually a long-term one.

Research on this topic in recent years presents irritable bowel syndrome as a complex of recurrent digestive symptoms, which are normally associated with abdominal pain, changes in the frequency and consistency of stool, abdominal flatulence, incomplete defecation accompanied by pain sensation, changes in bowel transit with variations between constipation and diarrhea, bloating and gas, mucus in the stool (Gilkin Jr, 2005; Longstreth *et al.*, 2006; Drossman, 2016). Laboratory tests have been shown to be deficient over time, and in the absence of any structural damage or radiological, endoscopic, histological or biochemical changes in the intestinal mucosa, the diagnostic procedure may be delayed or difficult (Spiller *et al.*, 2007).

According to the latest classifications for gastroenterological diseases (Videlock and Chang, 2015), irritable bowel syndrome is considered to be a functional gastrointestinal disorder that includes several key intestinal symptoms. The most common clinical manifestations of irritable bowel syndrome are changes in bowel habits, defined mainly by changes in stool consistency (constipation, diarrhea, or impairment due to lack of an exact clinical cause, such as intestinal inflammation or infection) (Canavan, West and Card, 2014). Additional symptoms include abdominal pain and discomfort, which sometimes improves with defecation, which occurs in the absence of a pathological trigger.

As early as 1820, irritable bowel syndrome was documented as an occasional pain reported in the intestines accompanied by a disturbance of digestive capacity, along with flatulence and a feeling of suffocation (Powell, 1818). Nearly 30 years later, Cummings talks about the heterogeneity of symptoms, both constipation and diarrhea, present in the same person (Cumming, 1849).

In the first descriptions of the disease, the focus was on the visible passage of mucus in the stool (called colonic mucosal disease) (Clark, 1859), especially in the form of pseudomembranes or portions of the colonic lumen (Da Costa, 1871; White, 1905). When these descriptions did not acquire a clear significance in terms of the pathology of the disease, it became very clear that a broadening of the symptomatic horizon is necessary.

Although the severity of symptoms varies from patient to patient, their prevalence varies as well. At the same time, the evolution of the disease being one of an extremely variable, unpredictable

nature, most often of long duration, the phases of remission alternating with the phases of reappearance of the symptomatology. Due to the chronic nature of this condition, the cause of which has not yet been fully elucidated, in which emotional, dietary, medicinal or hormonal factors may promote or aggravate gastrointestinal symptoms, the treatment is mainly focused on symptoms, with the most frequent emphasis on a diet (Lembo and Bollom, 2015).

To support the diagnosis of irritable bowel syndrome, the symptoms of the disease must meet the ROME IV criteria) which highlights the disruption of gastrointestinal tract activity, but bring an essential novelty, namely the concept of disorders of the brain-gut axis (gut-brain axis) (Schmulson and Drossman, 2017).

However, according to ROME IV criteria, functional gastrointestinal disorders have been given a new definition. Thus, functional gastrointestinal disorders are disorders of the bowel-brain interaction, a broad group of gastrointestinal disorders that include common symptoms such as motility disorders, visceral hypersensitivity, impaired mucosal and immune function, impaired intestinal microbiota, and impaired central nervous system processing (Drossman, 2016).

Given the incidence of symptoms and not knowing the exact pattern of interaction between genetic and environmental components, irritable bowel syndrome supports several clinical variants, thus being classified according to the main feature. Thus, considering the consistency of stools, irritable bowel syndrome was classified into 4 subtypes:

1. irritable bowel syndrome with constipation (IBS-C) characterized by hard stools at least 25% of the time or soft or watery stools less than 25% of the time;
2. irritable bowel syndrome with diarrhea (IBS-D) characterized by soft or watery stools at least 25% of the time or hard stools less than 25% of the time;
3. mixed type - soft or hard chairs at least 25% of the time,
4. unclassified - both soft and hard stools less than 25% of the time (Longstreth *et al*., 1997).

Current theories about the etiology of irritable bowel syndrome involve a number of factors, including the bowel-brain axis, psychosocial factors, inflammation, post-infectious mechanisms, genetic predisposition variations, diet or hormonal fluctuations.

The molecular approach to irritable bowel syndrome starts with a series of biomarkers, but which are not involved in the direct diagnosis, but in differentiating this disease from other diseases. In this way, in addition to the eight-element immunological biomarker panel described by Mujagic and colleagues, he proposed and validated interleukin 1-beta, interleukin 6, interleukin 12p70, tumor necrosis factor α, chromogranin A, human beta-defensin 2 (HBD2) and calprotectin in a multi-test IBS panel, many studies have described biomarker differentiation (Mujagic *et al.*, 2016).On the other hand, there is a perspective of the study of the microbiome in irritable bowel syndrome, and of oxidative stress.

The fact that oxidative stress is involved in many common physiological pathways and also in highly incidental pathologies, such as gastrointestinal, nutritional, neurological, and psychiatric pathologies, is not new to research (Mesika and Reichmann, 2019). Because irritable bowel syndrome could be a problem with poor signaling pathways involving both gastrointestinal secretion and neuro-vegetative stimulation, this condition is no exception to the oxidative assumption of pathological mechanisms.

It is known that the nervous system is susceptible to damage caused by oxidative stress due to its lipid structures and low antioxidant content (Finkel and Holbrook, 2000). On the other hand, there

are many theories linking oxidative stress and its mechanisms to gastrointestinal disorders and especially to the mechanisms of irritable bowel syndrome.

In a recent review of animal models of irritable bowel syndrome, Wang and colleagues (Wang *et al.*, 2017) noted that the underlying pathogenic mechanisms of the condition remain ambiguous, although intestinal permeability, inflammation, visceral hypersensitivity increase, and the brain-gut interaction is altered and plays a key role. However, the most commonly used method of treating irritable bowel syndrome symptoms in animal models is exposure to stress. This may suggest that stress response pathways may be involved in the symptoms of irritable bowel syndrome, regardless of its subtypes. However, research efforts have confirmed that even in these animal models, molecular changes occur at systemic and local action levels. Therefore, it appears that animal models of irritable bowel syndrome demonstrate that clinical diagnosis and evaluation would not be sufficient in research and treatment of the condition.

Regarding the involvement of oxidative stress in irritable bowel syndrome, previous research efforts have shown controversial results. While some animal models and patient studies have reported a clear oxidative imbalance at both systemic and local level, no concrete evidence indicates a direct correlation between oxidative stress and irritable bowel syndrome.

The correlation between the antioxidant system and irritable bowel syndrome has been further established in animal model studies. Thus, the link between inflammation and growth of reactive oxygen species (ROS) was made in a study that used a mixture of antioxidant potential of *Aloe vera* and *Matricaria recutita*, which aimed to describe its beneficial effects on induced gastrointestinal imbalance of restraint stress (Asadi-Shahmirzadi *et al.*, 2012).

Moreover, they observed that the administration of the mixture of plants and spasmolytics not only changed the imbalance of oxidative stress in colon cells, but also improved the antioxidant capacity, but without any evidence of the type of correlation between harmful events and antioxidant defense.

CHAPTER 2. RESEARCH MATERIALS AND METHODS

2.1. Research materials

2.1.1. Animals and animal models

The animals were treated according to the current legal framework, the experiments being performed in accordance with the legislation of Romania and the European Union regarding the use of animals in biomedical experiments. Every effort has been made to reduce the number and suffering of all animals used in the experimental models. At the same time, the experiments had the favorable opinion of the Ethics Commission of the Faculty of Biology within the Alexandru Ioan Cuza University of Iași and of the Ethics Commission of the Faculty of Veterinary Medicine, "Ion Ionescu de la Brad" University of Life Sciences, Iași.

The experimental studies conducted in the research focused on male Swiss mice with an initial body weight of 30-40 g and Wistar female rats with an initial body mass of 180-220 g.

In order to evaluate the behavioral aspects related to irritable bowel syndrome, we induced the symptoms of the condition through a series of experimental models, both in mice and rats. The experiments went in 3 different directions. A first experiment looked at the effect of multifactorial stress on a model of irritable bowel syndrome induced in mice. The second experiment was also carried out on mice subjected to multifactorial stress, but we carried out the research further out of the desire to evaluate the antioxidant effect of some ethanolic and methanolic extracts from *Camelina sativa* seedling, Mădălina variation. In the third experiment, we demonstrated the antioxidant capacity and cognitive relevance of the polyphenolic extract of *Chrysanthellum americanum* in an experimental animal model of irritable bowel syndrome in rats, also obtained by exposure to multifactorial stress.

The paradigm of multifactorial stress in mice

To obtain the experimental model based on the paradigm of multifactorial stress, 40 male mice were selected and four groups were created (n = 10). Three groups were subsequently exposed to different combinations of stress - as described below - and established as animal models of irritable bowel syndrome. The remaining group served as a control group and was subjected to identical environmental conditions in the absence of any studied stressors.

Two of the three IBS groups (n = 20) underwent neonatal separation (NMS) for 3 hours / day between postnatal days 1 and 14. The third IBS group and the control group remained untouched.

Starting postnatal day 90, the third IBS group and one of the two groups that underwent maternal neonatal separation (NMS) underwent 7 days (between 90 and 96) of multifactorial exposure to combined stress consisting of low-intensity stressors, unpredictable and a repetitive stressor.

During the 7 days of exposure, unpredictable low-intensity stressors were applied in the morning - except for lack of food / water, these stressors persisted for a period of 24 hours. In the second part of the day, the mice were subjected for 1 hour / day to the repetitive stress represented by the water avoidance stress paradigm.

Table 2.1 Type of stressors applied in each experimental group

Group 1 (MS)	Group 2 (MF)	Group 3 (MS+MF)	Group Control
maternal separation	multifactorial stress a. unpredictable (1) restraint stress (2) predator sound (3) water deprivation (4) injection simulation (5) tilt cage (6) tail pinch (7) food deprivation b. repetitive daily water avoidance stress	maternal separation multifactorial stress a. unpredictable (1) restraint stress (2) predator sound (3) water deprivation (4) injection simulation (5) tilt cage (6) tail pinch (7) food deprivation b. repetitive daily water avoidance stress	

After exposure to stress, all animals were subjected to behavioral assessment between days 101 and 109 in the following order: Y-maze test (day 101), plus maze test (day 103) and forced swimming test (days 106 and 109). 109). The collection of biological samples took place on day 111, as can be seen in Figure 2.1.

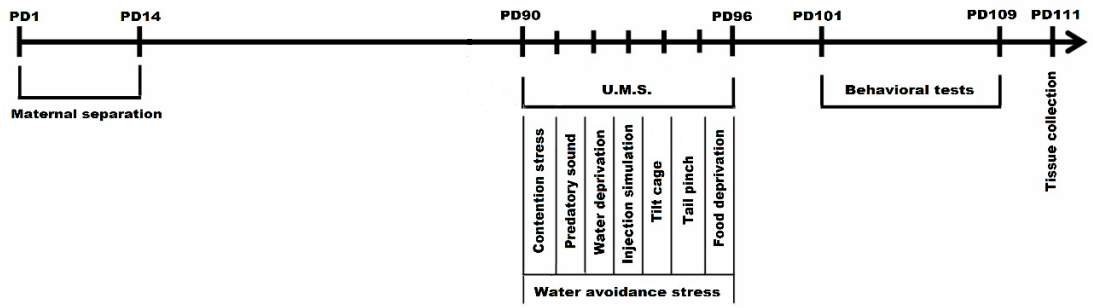


Figure 2.1. The experimental design of the irritable bowel syndrome model based on the multifactorial stress paradigm in mice.

The paradigm of multifactorial stress in mice and the administration of *Camelina sativa* extract

In order to induce irritable bowel syndrome symptoms in mice through an experimental model of chronic stress exposure, 36 newborns underwent maternal neonatal separation between postnatal days 1 and 14, and 3 days of restraint stress between postnatal days 90 and 92, for 30 minutes each day of the experiment. Exposure to contention stress consisted of immobilizing the mouse in a small cage which, although it provides the necessary space to live, does not allow it to move. Between days 93 and 98, the animals were exposed to a number of unpredictable, low-intensity, heterogeneous stressors:

1) stress factor by sound exposure of the predator for 10 minutes (the room that houses the animals is exposed to noises that mimic the sound of birds of prey);

2) stress factor by water deprivation for 24 hours;

3) stress factor by imitating an abdominal injection, according to the method of execution, but in the absence of the needle from the syringe;

4) stress factor caused by the inclination of the cage at an angle of 45 degrees to the horizontal plane between 1 and 4 hours;

5) stress factor obtained by pinching the tail, 1 cm from its end, with a pair of pliers for 1 minute;

6) stress factor by deprivation of food for 24 hours;

7) stress factor by restraint, during which the animal was immobilized for one hour a day in a small box.

An advantage of this approach, which aims to use heterogeneous stressors, is to avoid accustoming the animals, which is impossible to achieve in the conditions of constant application of homotypic stimuli.

In the period between 93 and 102, in addition to the heterogeneous stressors applied every day, the animals were subjected to an additional stressful stimulus, namely water avoidance stress. This procedure consisted of placing each mouse for one hour, on a platform with a diameter of 2.5 cm, in the middle of a small plastic pool, filled with water heated to 22^{o}C, at the height of the platform.

No stressors of any kind were applied to the animals in the control group, they were kept in controlled environmental conditions, receiving food and water *ad libitum.*

Between the days of 98 and 101, some mice received a dose of methanolic (ME) and ethanolic (EE) extract of *Camelina sativa*, respectively, to monitor its antioxidant effect, in doses of 5 g / kg body mass. The extracts were administered orally in the form of a suspension of 0.1% saline, a maximum of 0.5 ml of suspension per administration, using a gavage needle, according to standard procedures. The animals in the control group received the same dose of saline in order to ensure identical hydration conditions for all animals in the study.

In the period between day 102 and 110, we evaluated the animals from a behavioral point of view, and on day 112 they were slaughtered. We collected brain and gut samples for biochemical testing. Coprocytograms were made from the faeces.

To make the experimental model, the mice were divided into 4 subgroups, according to the figure below:

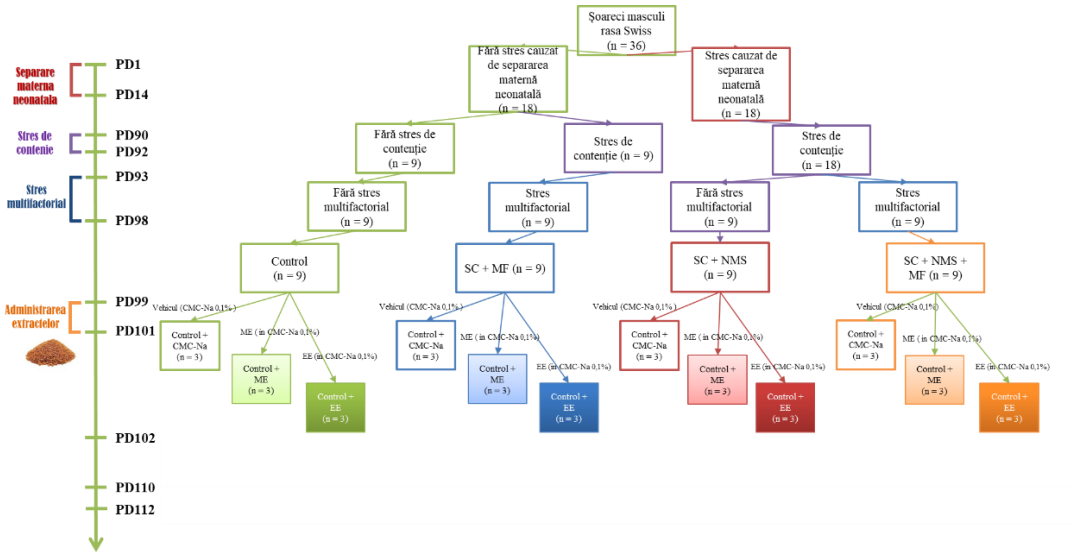


Figure 2.2. Schematic description of the experimental design targeting the multifactorial stress paradigm and administration of Camelina sativa extract

The paradigm of multifactorial stress in rats and the administration of *Chrysanthellum americanum* extract

To induce the symptoms of irritable bowel syndrome in rats, we used an experimental model that includes the multifactorial stress paradigm combined with a suite of different stressors, as described in some studies in the literature (Li *et al.*, 2016). For the current experiment, we adapted the method using an original combination of stressors to optimize the protocol and reduce the exposure time, which we presented earlier (LEFTER, Radu, CIOBICA, Alin, TIMOFTE, Daniel, ABABEI, Daniela, DOBRIN Dobrin, LUCA, Andrei, TRIFAN, Anca, STANCIU, Carol, SFARTI, Catalin, 2018).

Thus, IBS groups were exposed to two stressors each day for a relatively short period of only seven days. The first stressor was used constantly every morning, and the use of the second stressor, different every day, managed to minimize the habit of the animals.

The rats were exposed for one hour in the first half of the day, for a whole week, to the stress of avoiding water; the standard procedure is to place the rat on a platform measuring 8x6 cm, in the middle of a small plastic basin filled with warm water (25oC), at the height of the platform (Yang *et al.*, 2006). The rats of the control group were placed on the same platform, also for one hour, but in an empty container, without water.

One hour after the end of the episode that caused the water avoidance stress, the IBS group was exposed to one of 6 different stressors for each of the last six days of the protocol as follows:

1. Exposure to the sound of a predator for 5 minutes;
2. Deprivation of water for 24 hours;
3. Imitation of an abdominal injection;
4. Placing in a cage inclined at an angle of 45 degrees for 12 hours;
5. Pinching the tail, 1 cm from its end, with pliers for 1 minute;
6. Deprivation of food for 24 hours (Lü *et al.*, 2009).

Following the completion of this protocol, the polyphenolic extract of *Chrisanthellum americanum* was administered, followed by a day of rest and a battery of behavioral tests. The extract was administered orally, by gavage, for 5 consecutive days.

The 24 rats participating in this experiment were divided into 4 groups, each group having 6 animals, as follows:

- the control group received saline (2.5 ml / kg body weight) consecutively for 6 days;
- a group treated with polyphenolic extract of *Chrysantellum americanum* (100mg / kg body weight, for 6 consecutive days;
- a group in which irritable bowel syndrome was induced and which received saline (2.5 ml / kg body weight) 2 days during and 4 days after the rats were exposed to the multifactorial stress paradigm;
- a group in which irritable bowel syndrome was induced, but who was given the mentioned polyphenolic extract (100 mg / kg body weight) 2 days during and 4 days after the rats were exposed to the paradigm of multifactorial stress.

The dry polyphenolic extract was dissolved in saline (0.9 mg / ml) and administered intragastrically, by oral gavage on an empty stomach, one hour before any paradigm of exposure to stress.

2.1.2 Human patients

As the study on animal models followed 3 research paths, the study on human participants went in 2 directions which involved, in the first instance, the recruitment of patients and healthy volunteers.

In the first study, 10 patients with irritable bowel syndrome and 14 healthy volunteers from the Oftaprof Ophthalmic Clinic (Iasi, Romania) were recruited, with an average age of 42.6 years and a sex ratio of 50% women and 50% men. The patients recruited were divided into subgroups according to the classification system of irritable bowel syndrome: IBS-D (predominantly irritable bowel syndrome with diarrhea) and IBS-C (predominantly irritable bowel syndrome with constipation).

For the selection of the 14 healthy participants, we observed the sex ratio (50% women and 50% men), the average age being 39.42 years.

For the second study, 60 volunteers were recruited from St. Spiridon Hospital in Iasi, 15 of them being part of the control group, while the other 45 were divided into 3 subgroups, related to the classification system of the condition studied: 15 participants in the IBS-D subgroup (predominantly irritable bowel syndrome with diarrhea), 15 participants in the IBS-C subgroup (predominantly irritable bowel syndrome with constipation) and 15 participants in the IBS-M subgroup (irritable bowel syndrome) with mixed manifestations). The mean age for the control group was 43.72 years, for the IBS-D group 48 years, for the IBS-C 44.8 years, and for the IBS-M group 47.2 years. For all 4 subgroups, the ratio of 30% men and 70% women was maintained.

Prior to the actual involvement, patients were informed of the study in which they were included. They were presented and explained in detail the directions and stages of the experiment, information which can also be found in the Written Informed Consent, and the agreement expressed by the signature was given after clarifying the possible ambiguities.

Participants have been informed that they have the right to know the status of the study to which they belong, and will be contacted in order to obtain this information if they wish.

The study was conducted in accordance with national and European regulations on biomedical research and was approved by the local committee. The identity of the participants and personal data

were protected in accordance with the current legislation, using notations, IDs, meaningful to the investigator.

Obtaining and preparing biological samples

For the first experimental study, tear samples were collected in the morning on Schirmer strips according to the Schirmer test procedure without anesthesia (Stuchell et al., 1984), as presented by our research group (Balmus et al., 2020). For the second experimental study, the biological samples used consisted of venous blood, urine and feces.

2.2. Research methods

2.2.1. Behavioral determinations in experimental animals

Behavioral changes in the cognitive or affective-emotional sphere were highlighted after performing behavioral tests, in order to reach high thresholds of anxiety and depression in experimental animals. The methods can be found in "Methods of BehaviorAnalysis in Neuroscience" (Buccafusco, 2009).

2.2.1.1. Y maze test

The basis of this paradigm consists in the native tendency of the animal to explore the environment in which it is, respectively the successive and alternating exploration of the three arms that enter the structure of the device, thus evaluating its short-term memory.

2.2.1.2. Elevated plus maze test

Elevated plus maze test is used to assess anxiety in experimental animals.

2.2.1.3. Forced swimming test

Forced swimming test is a commonly used method for observing the depressive behavior of laboratory animals.

Figure 2.3. Y maze test

Figure 2.4. Elevated plus maze test

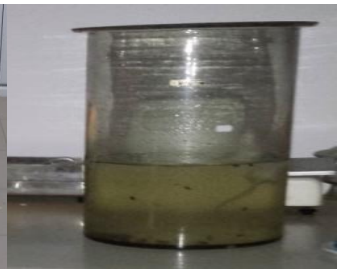


Figure 2.5. Forced swimming test

2.2.2.Behavioral assessment of human participants

2.2.2.1. Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS)

The VAS-IBS Analog Visual Scale is a well-known pain assessment scale originally developed by Prof. Dr. Mariette Bengtsson in 2007. Based on the diagnostic criteria for ROME III gastrointestinal disease, Prof. Bengtsson and colleagues proposed VAS-IBS as a tool that could be used in clinical practice by various healthcare professionals to assess the condition over time of patients with IBS (Bengtsson *et al.*, 2011).

However, even though Bengtsson and colleagues suggested that the English version of VAS-IBS be used in English-speaking countries and be further tested for validity and reliability in English-speaking patients (Bengtsson et al. , 2013), the Romanian population is not a predominantly English-speaking population. Thus, in court, we set out to validate the translation and adaptation in Romanian of the VAS-IBS scale in accordance with the ROME IV criteria, and in this way we thank for the goodwill and good collaboration with the author of this scale.

Thus, according to Schmulson and Drossman (Schmulson and Drossman, 2017), the ROME IV criteria have been updated, including changing "last month" to "≥ 1 day / week in the last 3 months" for pain, and eliminating "abdominal discomfort" due to non-specific and ambiguous expression in many languages. Also, differentiation between IBS subtypes is recently based on the Bristol scale for at least 25% of bowel movements. In this way, the translated and adapted VAS-IBS was applied in the form of a completion questionnaire offering a possibility to answer from a scale of 1 to 10 (Figure 2.7).

The translation was performed by specialists in gastroenterology, psychiatry and physiology and revised by a native English speaker (Dr. Walter Bild, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, Romania). After the translation, the questionnaires were completed by 14 healthy people (7 men, 7 women) to assess their understanding. No further updates required. We also encourage Romanian gastroenterology clinicians to use this scale on a wide variety of patients with gastrointestinal disorders to check and evaluate the usefulness, strengths and weaknesses of our translation.

2.2.2.2. *Pittsburgh Sleep Quality Index Questionnaire*

The Pittsburgh Sleep Quality Index (PSQI) available at http://www.goodmedicine.org was used to assess sleep disorders and patterns in patients with irritable bowel syndrome. uk /.

2.2.3. Biochemical and immunoenzymatic determinations

Biochemical and immunoenzymatic determinations performed to assess oxidative stress and inflammatory status in serum or other biological products were performed at the Faculty of Biology of Alexandru Ioan Cuza University in Iasi.

The biochemical determinations are based on colorimetric measurement techniques, and the spectrophotometric measurement system Specord 210 PLUS Analytik Jena was used to evaluate the absorbances.

Enzyme-linked immunosorbent assays were performed by ELISA technique, and the absorbances and analyte concentrations were obtained using the Mindray MR-96A automatic ELISA microplate reader, Shenzhen, PR China.

2.2.4. Evaluation and stained cytological examination of faeces

The coprocytogram is a series of tests performed on a sample of faeces in order to diagnose certain diseases of the digestive tract, but especially to assess the inflammatory response at its level. Spontaneously issued fresh faeces are required in a faecal container with no transport medium. Preferably, the sample is examined on the same day as the harvest due to low stability (maximum 24 hours at 2-8ºC).

With a handle, enough contents were taken to be spread on the surface of a blade, after which it was left to dry at room temperature. A number of drops of May - Grunwald solution was placed over the blade on the staining stand, enough to cover the entire smear. The fixation was done with methyl alcohol, after which the blade was covered with diluted Giemsa solution.

After washing and drying the slides, microscopic examinations were performed. The reading took place under the Nikon Eclipse E200 microscope, at the 100X lens with immersion oil and a qualitative assessment was made (very rare, rare, present, relatively frequent and frequent). After reading, the slides were degreased with toluene and stored in the histotheque.

2.2.5. Statistical interpretation of results

The results obtained in the presented studies were statistically analyzed using Minitab 19 software (Minitab Inc., 2019). One-way variance analysis was used to assess the significance of variance between and in groups, as well as the Student's t test. All results were expressed as mean ± S.E.M. Statistical correlations were also calculated using the Pearson and Spearman coefficients. F values for which $p < 0.05$ were considered statistically significant.

CHAPTER 3. RESULTS AND DISCUSSIONS

3.1. Results obtained by studying animal models

3.1.1. Results from the application of the multifactorial stress paradigm to mice

3.1.1.1.1. The effect of different combinations of stressors on the gastrointestinal tract

The assessment of gastrointestinal tract habits in experimental animals provided important information on the effect of multifactorial stress, so we observed that exposure to different combinations of stressors can induce significant increases in intestinal transit time. A tendency to constipate the animals was also identified in all three combinations of stressors compared to the control group, this being quantified by the number of feces obtained in 24 hours (Figure 3.1). However, the larger differences from the control group were clearer for the group exposed to multifactorial stress and neonatal maternal separation, NMS + MF vs. control ($F(1, 18) = 24.13$, $p = 0.001$).

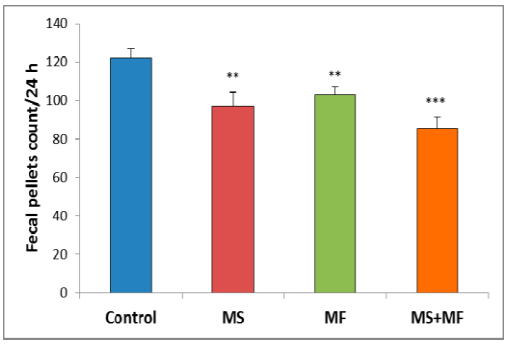


Figure 3.1. The effect of different combinations of stressors on the habits of the gastrointestinal tract, as shown by the number of feces per 24 hours. Values are mean S.E.M (n = 10 per group, ** $p < 0.01$, *** $p < 0.001$, NMS = neonatal maternal separation, MF = multifactorial stress.

3.1.1.1.2. Effects of different combinations of stressors on the parameters assessed using the Y maze test

Behavioral analysis of cognitive performance, in this case short-term memory, did not show statistically significant general differences between the experimental groups ($F(3, 36) = 2.28$, $p = 0.09$) in terms of the percentage of alternation spontaneous. However, the post-hoc analysis indicated a statistically significant decrease in the percentage of spontaneous alternation in the MF group ($F(1, 18) = 4.42$, $p = 0.04$; $t(18) = 2.11$, $p = 0.02$) and the NMS + MF group ($F(1, 18) = 5.65$, $p = 0.02$; $t(18) = 2.03$, $p = 0.03$), compared to the control group (Figure 3.2).

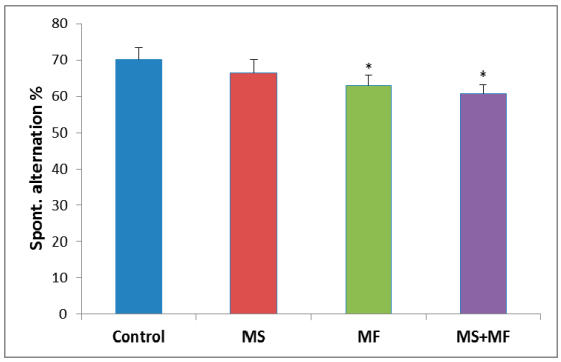


Figure 3.2. The effects of different combinations of stressors in the Y maze test, as shown by the spontaneous alternation parameter. Values are mean ± S.E.M (n = 10 per group, * p <0.05 vs. control, NMS = neonatal maternal separation, MF = multifactorial stress.

3.1.1.1.3.Effects of different combinations of stressors on the parameters evaluated using the elevated plus maze test

To assess anxiety in experimental animals, the number of entries in the open arms of the maze was quantified, which is a parameter whose low values are characteristic of anxiogenic behavior, based on the natural aversion of rodents to open rooms. The number of open arms entries visibly decreased in all groups exposed to stress, but statistically significant decreases were recorded in NMS + MF vs. control ($F(1, 18) = 6.18$, $p = 0.023$; $t(9) = 2.75$, $p = 0.01$) and almost significant in the NMS vs. control group ($F(1, 18) = 3.92$, $p = 0.063$; $t(16) = 1.97$, $p = 0.03$) (Figure 3.3).

Time spent in the open arms (Figure 3.4), one of the most suggestive indicators for anxiolytic effects, decreased visibly in all groups exposed to stress compared to the control group, but a statistically significant decrease was observed only for groups MF vs. control group ($F(1, 18) = 4.52$, $p = 0.047$; $t(10) = 2.12$, $p = 0.02$) and MS + MF vs. control group ($F(1, 18) = 4.42$, $p = 0.049$; $t(18) = 2.10$, $p = 0.03$).

An interesting aspect was observed when we analyzed the exploration of the closed arms (Figure 3.5), a parameter oriented to the locomotor function, and we found a significant decrease in the number of entries in the closed arms of the labyrinth in the NMS group ($F(1, 18) = 5.44$, $p = 0.037$; $t(16) = 2.33$, $p = 0.01$) compared to the control group, while the other two groups exposed to stress showed less significant decreases.

Grooming episodes (Figure 3.6), another indicator used to assess anxiety, did not show any statistically significant overall variation between groups, except for a moderate increase in the NMS group ($F(1, 18) = 4.64$, $p = 0.044$, $t(12) = -2.15$, $p = 0.01$, compared to the control group.

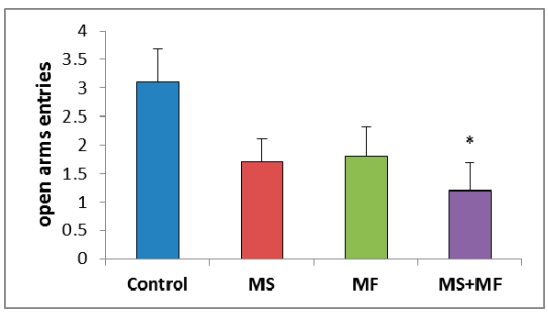


Figure 3.3. The effects of different combinations of stressors on the number of open arms entries evaluated in elevated plus maze test. Values are mean ± S.E.M (n = 10 per group, * p <0.05 versus control, NMS = neonatal maternal separation, MF = multifactorial stress)

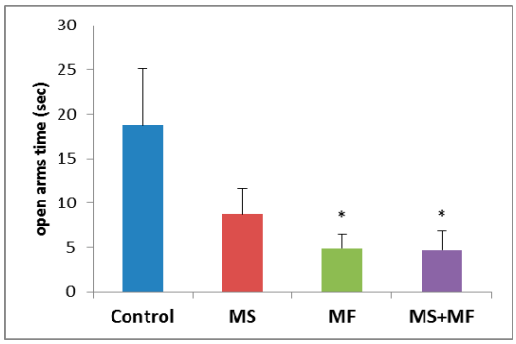


Figure 3.4. The effects of different combinations of stressors on the time spent in the open arms evaluated in elevated plus maze test. Values are mean ± S.E.M (n = 10 per group, * p <0.05 versus control, NMS = neonatal maternal separation, MF = multifactorial stress)

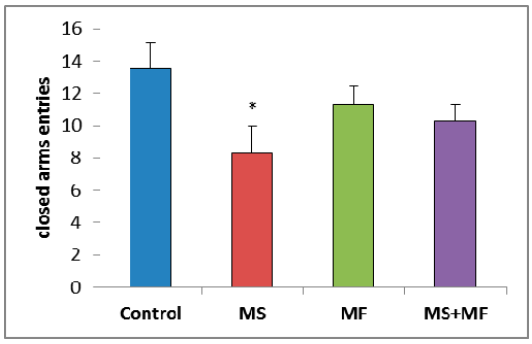


Figure 3.5. The effects of different combinations of stressors on the number of entries in the closed arms evaluated in elevated plus maze test. Values are mean ± S.E.M (n = 10 per group, * p <0.05 compared to control, NMS = neonatal maternal separation, MF = stress multifactorial)

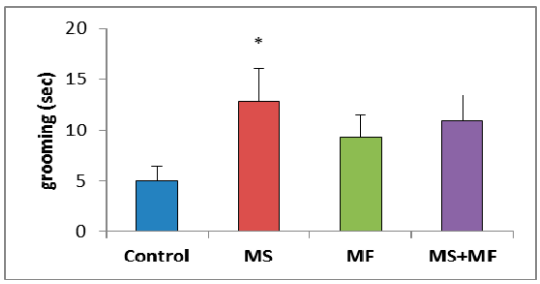


Figure 3.6. The effects of different combinations of stressors on grooming episodes assessed in elevated plus maze test test. Values are mean ± S.E.M (n = 10 per group, * p <0.05 versus control, NMS = neonatal maternal separation, MF = multifactorial stress).

3.1.1.1.4. Effects of different combinations of stressors on the parameters assessed using the forced swimming test

For the forced swimming test, the statistical analysis of swimming time showed a statistically significant decrease for the NMS group compared to the control group (F (1, 18) = 6.69, p = 0.018; t (17) = 2.58 , p = 0.009), although swimming time was visibly low in the other two groups exposed to stress (Figure 3.7).

Post-hoc analysis showed significant overall differences between groups for immobility time (floating) (F (3, 36) = 2,874, p = 0.04). Clearly, downtime increased in all three stress groups, with a statistically significant increase in the multifactorial stress group, MF, versus the control group (F (1, 18) = 3.64, p = 0.048; t (18) = 2.10, p = 0.02) and, respectively, the group exposed to multifactorial stress and neonatal maternal separation, NMS + MF, (F (1, 18) = 6.614, p = 0.019; t (14) = 2.57, p = 0.01) compared to the control group (Figure 3.8).

Moreover, the tests revealed a significant overall difference between the groups in terms of the time of struggle (F (3, 36) = 5.433, p = 0.03) and a significant decrease in the NMS + MF group compared to the group. control (F (1, 18) = 7.154, p = 0.015; t (13) = 2.67, p = 0.009) and also to

the MS group (F (1, 18) = 4.21, p = 0.05; t (10) = 3.62, p = 0.02) (Figure 3.9). Interestingly, we observed an almost significant increase in throbbing time, which may suggest anxious behavior in the multifactorial stress group compared to the control group (F (1, 18) = 3,928, p = 0.06; t (13) = −1.98, p = 0.03).

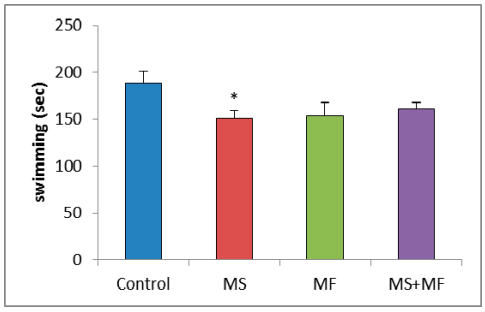


Figure 3.7. The effect of different combinations of stressors on swimming time assessed in the forced swimming test. Values are mean ± S.E.M, n = 10 per group, * p <0.05 versus control and # p <0.05 versus NMS group (NMS = neonatal maternal separation, MF = multifactorial stress).

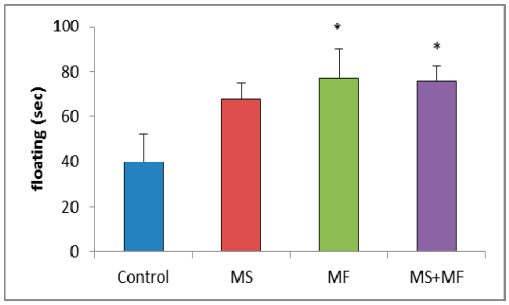


Figure 3.8. The effect of different combinations of stressors on immobility time (floating) assessed in the forced swimming test (c) fighting time (seconds). Values are mean ± S.E.M, n = 10 per group, * p <0.05 versus control and # p <0.05 versus NMS group (NMS = neonatal maternal separation, MF = multifactorial stress).

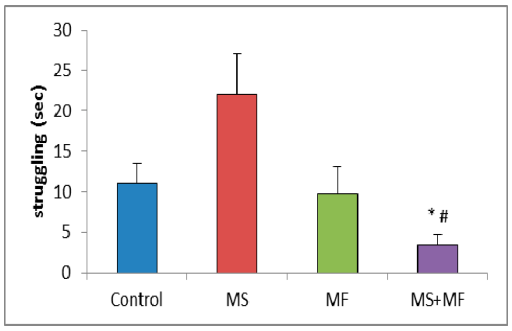


Figure 3.9. The effect of different combinations of stressors on the beating time assessed in the forced swimming test (c) fighting time (seconds). Values are mean ± S.E.M, n = 10 per group, * p <0.05 versus control and # p <0.05 versus NMS group (NMS = neonatal maternal separation, MF = multifactorial stress).

3.1.1.2. Evaluation of biochemical parameters present in brain tissue

The evaluation of the oxidative stress parameters, in this case the post-hoc analysis, did not show statistically significant variations between groups in terms of antioxidant activity of SOD (Figure 3.10), although enzymatic activity showed visible decreases mainly in the NMS + group. MFvs. control (F (1, 18) = 4.27, p = 0.53; t (18) = 2.06, p = 0.02) and slight increases in the group exposed to multifactorial stress (MF).

However, GPx levels were generally low in the stress-exposed groups and significant decreases were detected in the MF and NMS + MF groups compared to the control group (F (1, 18) = 15.33, p = 0.002; t (12) = 3.91, p = 0.002) and (F (1, 18) = 8.44, p = 0.009; respectively t (17) = 2.90, p = 0.04) (Figure 3.11).

Regarding the concentration of MDA in the brain tissue, significant general differences were observed between groups (F (3, 36) = 3.21, p = 0.03), indicating the effect of stress exposure on this parameter of oxidative stress. Elevated levels of malondialdehyde were observed in the MF group

compared to the control group (F (1, 18) = 8,604, p = 0.008; t (15) = 2.93, p = 0.005) and NMS + MF compared to the control group (F (1, 18) = 7.516, p = 0.013; t (14) = -2.74, p = 0.007), but not in the NMS group (Figure 3.12).

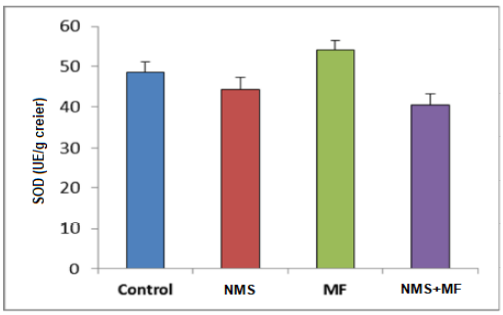


Figure 3.10. The effect of different combinations of stressors on brain SOD activity in mice. Values are mean ± S.E.M. (n = 10 per group, * p <0.05 and ** p <0.01, NMS = neonatal maternal separation, MF = multifactorial stress).

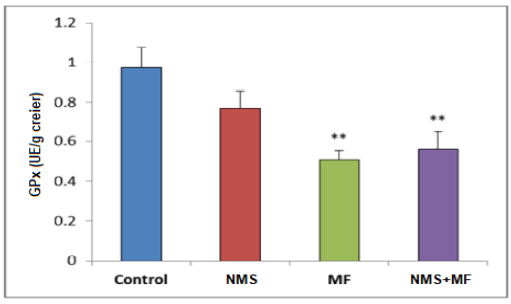


Figure 3.11. The effect of different combinations of stressors on GPx activity in the brain in mice. Values are averages ± S.E.M. (n = 10 per group, * p <0.05 and ** p <0.01, NMS = neonatal maternal separation, MF = multifactorial stress).

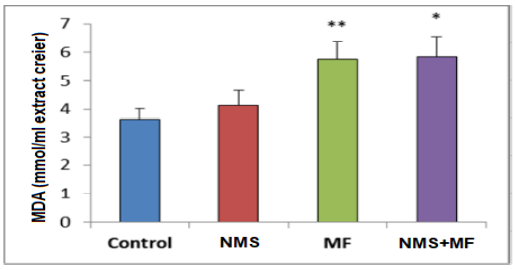


Figure 3.12. The effect of different combinations of stressors on MDA levels in the brain in mice. Values are averages ± S.E.M. (n = 10 per group, * p <0.05 and ** p <0.01, NMS = neonatal maternal separation, MF = multifactorial stress)

3.1.1.3. Evaluation of biochemical parameters present in intestinal tissue

Regarding markers of oxidative stress at the colonic level, there are no significant differences in SOD activity between groups (Figure 3.13).

Similarly, the enzymatic activity of GPx in the stressed groups did not vary significantly from the control, except for a statistically significant decrease in the NMS group versus the control group (F (1, 18) 7.77, p = 0.012; t (18) = 2.78, p = 0.006) (Figure 3.14).

Intestinal tissue MDA levels increased statistically significantly in the NMS + MF group compared to the control group (F (1, 18) = 7.241, p = 0.0149; t (15) = 2.69, p = 0.008), while the other two groups exposed to stress did not have significant increases compared to the controls (Figure 3.15).

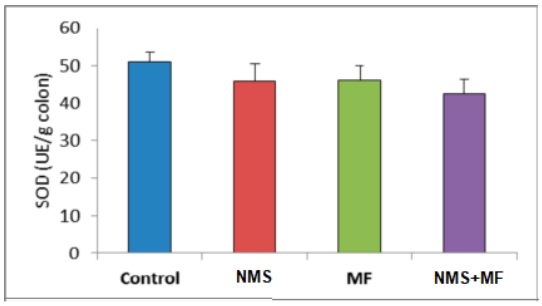


Figure 3.13. The effect of different combinations of stressors on colonic SOD activity in mice. Values are mean ± S.E.M. (n = 10 per group, * p <0.05 and ** p <0.01, NMS = neonatal maternal separation, MF = multifactorial stress).

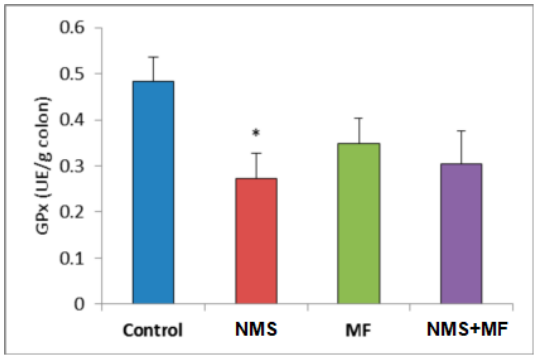


Figure 3.14. The effect of different combinations of stressors on colonic GPx activity in mice. Values are mean ± S.E.M. (n = 10 per group, * p <0.05 and ** p <0.01, NMS = neonatal maternal separation, MF = multifactorial stress).

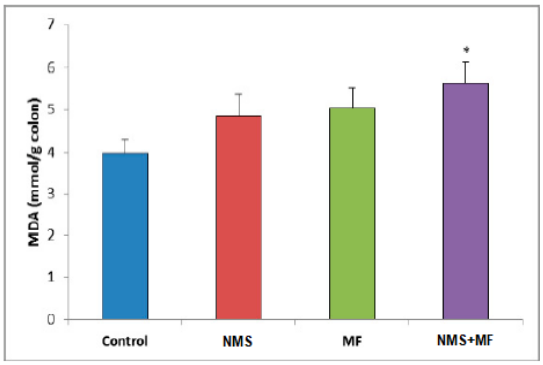


Figure 3.15. The effect of different combinations of stressors on MDA levels in the brain in mice. Values are averages ± S.E.M. (n = 10 per group, * p <0.05 and ** p <0.01, NMS = neonatal maternal separation, MF = multifactorial stress).

3.1.2. Results from the application of the multifactorial stress paradigm to mice and administration of *Camelina sativa* extract

3.1.2.1.1. The effect of the administration of the methanolic and ethanolic extract of *Camelina sativa* var. Madalina on short-term memory

Regarding the assessment of short-term memory in experimental mice using the Y-maze test, there were no statistically significant overall differences between the groups exposed to stress and treated with methanolic and ethanolic extracts (ME and EE), but we were able to highlight a significant general variation related to the percentage of spontaneous alternation (F 11, 24) = 3.89, p = 0.000874), even if we could not observe significant differences in relation to locomotor activity (F 11, 24 = 1.18, p = 0.331).

Subsequent analysis of the data showed that there were no significant variations between the unstressed control group that received the methanolic extract (p = 0.646) or the unstressed control group that received the ethanolic extract (p = 0.646) compared to the control group (Figure 3.30).

Also, when we compared the effect of the two extracts from the groups exposed to stress, respectively, controls, we did not observe statistical differences or variations between the control +

EE and control + ME groups (p = 0.817). However, there was a tendency to improve short-term memory in the group exposed to multifactorial stress and restraint (SC + MF) that received EE, compared to ME (p = 0.018) and saline (p = 0.007). Similarly, EE has been shown to be more effective in improving short-term memory in the SC + NMS + MF stress group of mice compared to ME (p = 0.05).

Also, a significant increase in short-term memory performance was observed in the group of mice exposed to stress caused by neonatal maternal separation and restraint stress (SC + NMS) while receiving EE (p = 0.048), compared to the corresponding but untreated stressed group, and no significant difference between the effect of ME and EE on this type of stress (p = 0.116). However, it was observed that short-term memory performance was significantly lower in the group of stressed and ME-treated mice compared to the control group that received this extract (p = 0.008).

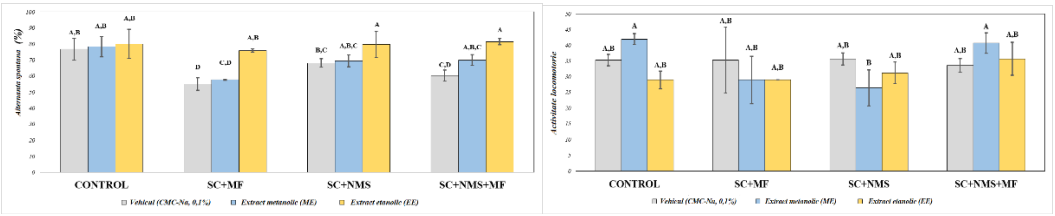


Figure 3.30. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from Camelina sativa var seeds. Madalina on short-term spatial memory (a) and locomotor activity (b), as observed in the Y maze test, in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract; A, B, C , D = Fisher's LSD test).

3.1.2.1.2. The effect of the administration of methanolic and ethanolic extract of *Camelina sativa* var. Mădălina on anxious behavior

Anxiety-like behavior was assessed using the raised maze test, and we found a significant group difference in the time spent in the open arms of the device (F 11, 24) = 6.04, p = 1.5E − 05) and the number of entries in open arms (F 11, 24) = 2.05, p = 0.0045). Subsequent analysis showed no difference in the occurrence of anxiety behavior in the unstressed groups while receiving ME (p = 0.719) and EE (p = 0.28), but there were significant changes in the number of open arms (therefore animal mobility) in the SC + MF group treated with ME vs. EE + treated SC + MF (p = 0.026) (Figure 3.31).

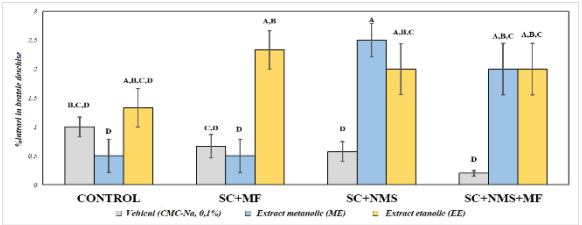


Figure 3.31. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from *Camelina sativa* var. Madalina on anxious behavior, as observed in elevated plus maze test, by the number of entries in the open arms, in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract; A, B, C , D = Fisher's LSD test).

The significant anxiolytic effect was also highlighted in the groups exposed to the three types of stress (SC + NMS + MF) receiving ME and EE (p <0.001) (Figures 3.31 and 3.32), but with a higher yield for EE (p <0.001). = 0.01, compared to ME) (Figure 3.32). A significant improvement in

anxiety behavior was observed in the SC + NMS group after administration of ethanolic extract (EE) (p = 0.034) and only a slight improvement in this behavior resulted after administration of the same extract in the SC + MF group (p = 0.07), as can be seen from the time spent in the open arms (Figure 3.32).

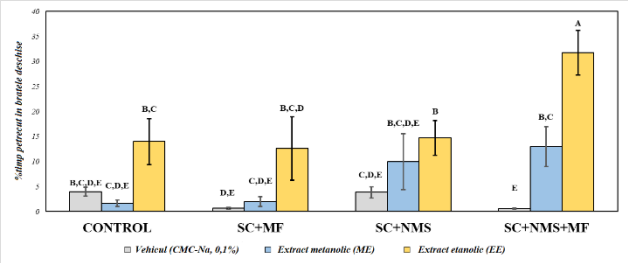


Figure 3.32. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on anxious behavior, as observed in the elevated plus maze test, through the time spent in open arms, in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract; A, B, C , D = Fisher's LSD test).

However, a statistically significant improvement was observed for both extracts administered to the group of mice most exposed to stress (p ME = 0.013, p EE <0.001), with anxiety-like behavior still significantly amplified for these. groups, compared to the control group (pME = 0.05, pEE = 0.014, control face + extracts).

Because it is also a significant parameter for assessing anxiety behavior, the number of entries in the closed arms of the device did not vary between the experimental groups (F 11, 24) = 2.03, p = 0.052) (Figure 3.33). Despite this, we observed that both ME (p = 0.045) and EE (p = 0.049) could show an anxiolytic effect when administered to the SC + NMS group. Similarly, we observed that the negative effects of stress on emotional status are significantly attenuated with the help of Camelina sativa var seed extract. Madalina in the groups SC + MF (pEE = 0.041), SC + NMS (pME = 0.003, pEE = 0.024) and SC + NMS + MF (pME = 0.004), despite the fact that there are also significantly different from the group control (pME = 0.04).

Therefore, by comparison, we observed that the methanolic extract was more effective in order to alleviate the anxiety behavior in the group exposed to restraint stress and neonatal maternal separation (p = 0.01) and in the group exposed to the three types of stress (SC + NMS + MF) compared to the SC + MF group (p = 0.004).

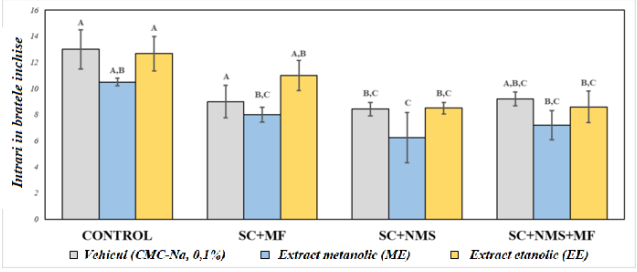


Figure 3.33. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on anxious behavior, as observed in the elevated plus maze test, by the number of entries in the closed arms, in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract; A, B, C , D = Fisher's LSD test).

Regarding the occurrence of anxiolytic behavior (grooming), we did not observe any significant general difference between groups (F 11, 24) = 0.26, p = 0.988) (Figure 3.34).

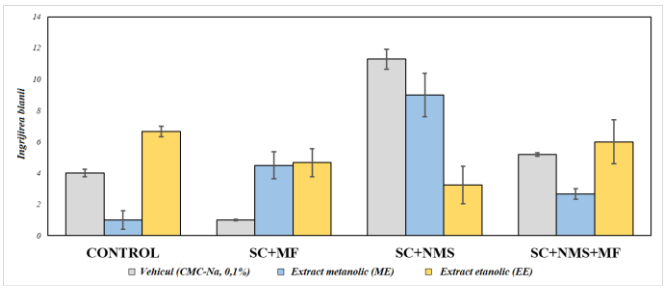


Figure 3.34. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on anxious behavior, as observed in the elevated plus maze test, through the grooming behavior, in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract).

3.1.2.1.3. The effect of the administration of methanolic and ethanolic extract of *Camelina sativa* var. Mădălina on depressive behavior

During the evaluation of the effects of the two studied seed extracts on the appearance of a depression-like behavior in the experimental model of irritable bowel syndrome in mice, we observed significant general changes in swimming time (F 11, 24) = 2.96, p = 0.006). Moreover, we found that there are statistically significant differences between the two extracts while being administered to unstressed mice (p = 0.031), while EE was considered less effective in preventing the occurrence of depressive behavior (p = 0.393). In addition, the positive effect of ME in terms of antidepressant potential was also observed in the group most exposed to stress (SC + NMS + MF) (p = 0.05), respectively SC + NMS (p = 0.011) (Figure 3.35) .

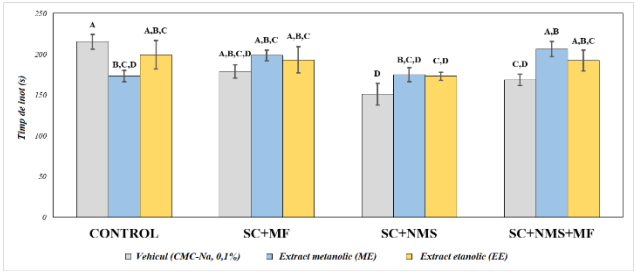


Figure 3.35. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on depressive behavior, as observed in the forced swimming test, through swimming time, in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B, C , D = Fisher's LSD test).

Regarding the time spent by the animal in immobility, no significant variations were identified between the studied groups (F 11, 24 = 2.34, p = 0.27). An antidepressant effect, visible and statistically significant of the methanolic extract, could be observed in the SC + NMS + MF group compared to the control group (p = 0. 002) (Figure 3.36).

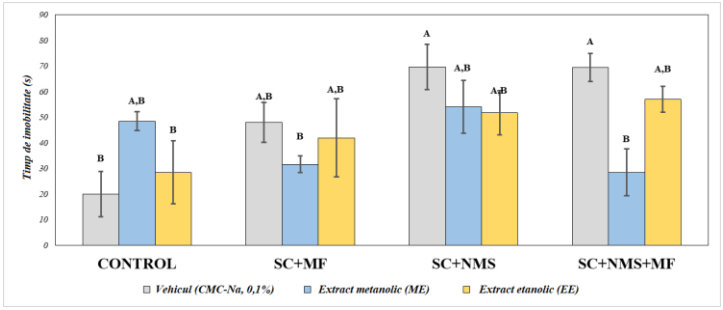


Figure 3.36. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on depressive behavior, as observed in forced swimming test, during the time of immobility, in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention

stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B = test Fisher's LSD).

The struggle behavior of the animals during the battery of behavioral tests revealed significant general differences between the studied groups (F 11, 24) = 2:0, p = 0.045). In this regard, we observed that EE had a better yield in decreasing the behavior similar to depression in the SC + MF group (p = 0. 049) and the SC + NMS + MF group (p = 0.013), compared to the mice in the group SC + NMS (Figure 3.37).

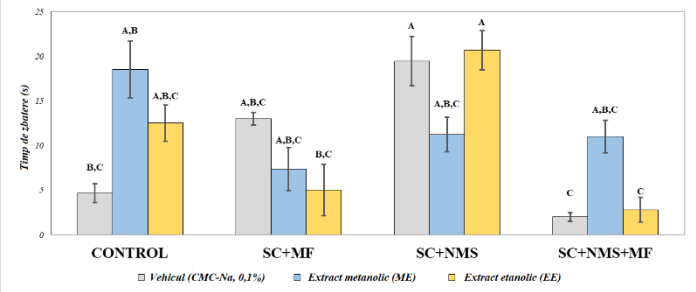


Figure 3.37. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on depressive behavior, as observed in forced swimming test, through the time of struggle, in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B, C = Fisher's LSD test).

3.1.2.2. The effect of methanolic and ethanolic extract from the seeds of *Camelina sativa* var. Madalina on the oxidative status of the brain tissue

Studying the activities of the enzymes involved in oxidative stress in the brain and their variation in the experimental model, we observed that both used extracts have a significant overall effect in terms of SOD activity, (F 11, 96) = 5.06, p = 2.39 E − 06) but without recording modulatory effects while administered to groups containing unstressed mice (pME = 0.22, pEE = 0.113, compared to the control group). It also became apparent that the largest difference in modulatory effect was obtained after the administration of extracts in the SC + NMS groups (p <0.001). Moreover, we observed that both ME and EE expressed a significant modulation of SOD activity while administered in the SC + MF group (pME <0.001, pEE <0.001, compared to the control group). and in the SC + NMS group (pME = 0.013, pEE = 0.007, compared to the control group) (Figure 3.38).

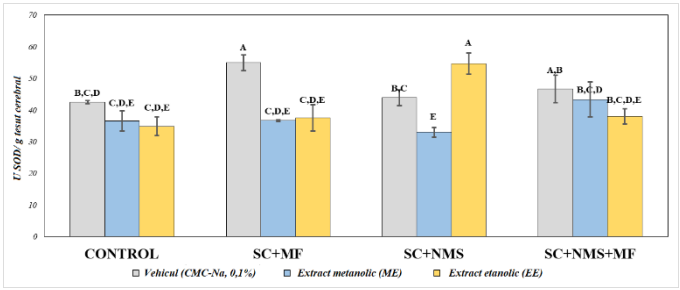


Figure 3.38. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on the activity of SOD in the brain in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B, C , D, E = Fisher's LSD test).

Analysis of the effect of extracts from the seeds of *Camelina sativa* var. Madalina on the specific activity of GPx, revealed significant general differences between the studied groups (F 11, 96) = 2.62, p = 0.005) and statistically significant differences between the modulatory potential of ME during administration in unstressed mice (p = 0.048), with diametrically opposite effects when administered to mice in the SC + NMS + MF group (p = 0.007).

The capacity and antioxidant effect of the ethanolic extract was observed mainly when administered to the group exposed to all three types of stress, SC + NMS + MF (pEE = 0.034) (Figure 3.39). Furthermore, it was observed that GPx activity in the brain tissue in mice treated with ME and EE from the SC + NMS groups (pEE = 0.043) and SC + NMS + MF (pME = 0.001) could exceed the levels present in the control group.

However, the methanolic extract administered to the SC + NMS + MF group (p = 0.05) has the highest yield in terms of modulating GPx activity in brain tissue (p = 0.05), while ethanolic extract was more effective in modulating GPx enzyme activity. in the SC + NMS group (p = 0.008).

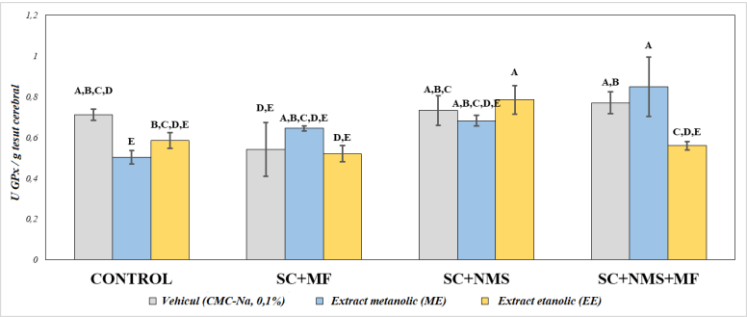


Figure 3.39. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on GPx activity in the brain in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B, C , D, E = Fisher's LSD test).

Biochemical analysis of the studied samples revealed that the extracts obtained from the seeds of *Camelina sativa* var. Madalina have a significant overall effect on MDA levels (F 11, 96) = 17.59, p = 1.7749E − 19), but no difference was observed in their effect on mice that were not exposed to stress (pME = 0.199, pEE = 0.261). Notably, statistically significant differences were identified in terms of their effect on MDA levels between the two types of extracts.

Thus, the differences were observed in the group exposed to restraint stress and that caused by neonatal maternal separation, SC + NMS (p = 0.015), in the group exposed to contention stress and multifactorial stress, SC + MF (p <0.001), and in the group exposed to all three types of stressors, SC + NMS + MF (p <0.001), having a more visible effect in the last group.

Regarding the efficiency of extracts with respect to lipid peroxidation in the brain, both extracts, both methanolic and ethanolic, had visible effects in the SC + MF group (pME <0.001, pEE = 0.013). At the same time, we noticed that the ethanolic extract significantly increased the lipid peroxidation process in the brain tissue in the SC + NMS group (p = 0.006). Moreover, our results suggested that methanolic extract may show inflammatory effects (SC + NMS + MF + ME vs. C + ME, p <0.001; SC + NMS + MF + ME vs. SC + NMS + MF, p <0.001), especially in the group most exposed to stress (Figure 3.40).

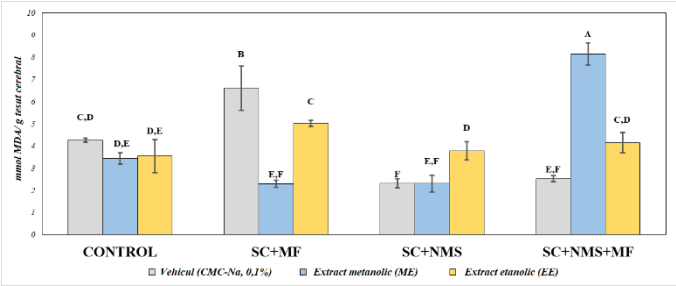


Figure 3.40. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on MDA levels in brain tissue in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B, C , D, E, F = Fisher's LSD test).

Regarding the total soluble protein levels in the brain tissues, when we analyzed the general effect of extracts of *Camelina sativa*, var. Madalina we found statistically significant differences between groups (F 11, 96) = 3.64, p = 0.00021) and significant differences between extracts when administered to the group of mice exposed to restraint stress and multifactorial (p = 0.002). At the same time, there were significant increases in the levels of total soluble proteins in the brain tissue after the administration of both types of extracts (pME = 0.037, pEE <0.001) (Figure 3.41).

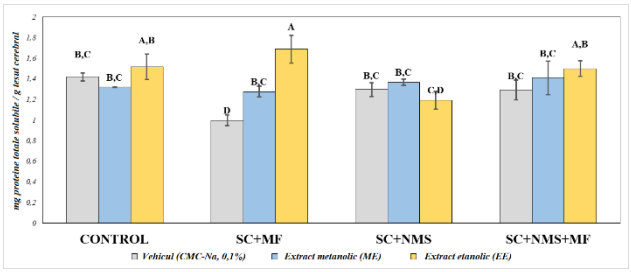


Figure 3.41. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on total soluble protein levels in brain tissue in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B, C , D, E, F = Fisher's LSD test).

3.1.2.3. The effect of methanolic and ethanolic extract from the seeds of *Camelina sativa* var. Madalina on the oxidative status of the colonic tissue

Our study also assessed the oxidative status of intestinal tissue after administration of the two types of extracts, methanolic and ethanolic, in the experimental animal model of irritable bowel syndrome in mice. In this respect, the results obtained indicated a statistically significant overall effect between groups of these extracts on SOD activity at the colonic level (F 11, 96) = 5.57, p = 5.1E − 07).

Comparing the effects of the two extracts, we did not notice any significant differences in the activity of the SOD enzyme, when they were administered to unstressed mice in the control group (pME = 0.385, pEE = 0.511). However, despite this, the results revealed statistically significant differences in the modulatory effect of the extracts on the enzymatic activity studied in this case in the SC + NMS (p = 0.014) and SC + MF (0.027) groups. , with a more efficient modeling effect in the latter group (pME <0.001, pEE = 0.027).

Analyzing this aspect, we found that an exposure to additional stress does not necessarily have a cumulative effect, especially since the activity of SOD in colonic tissues in animals in the group

SC + NMS + MF showed a low yield after administration of both extracts (pME = 0.047, pEE = 0.02) compared to the control group (Figure 3.42).

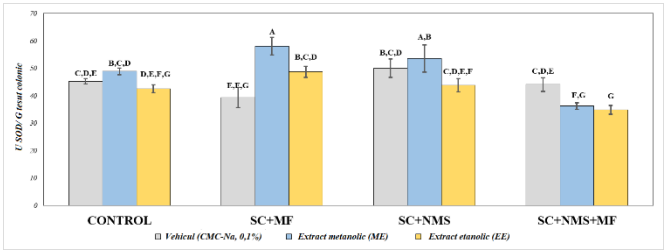


Figure 3.42. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on colonic SOD activity in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B, C , D, E, F, G = Fisher's LSD test).

The antioxidant potential of extracts from the seeds of *Camelina sativa* var. Madalina was also evaluated in terms of the modulatory effect they show on the specific activity of GPx in the mentioned tissues (F 11, 96) = 4.80, p = 5.36E − 06). Although no differences were identified from this point of view, after administration of extracts to non-stressed mice (pME = 0.377, pEE = 0.972), we observed that GPx activity decreased significantly after administration of ethanolic extract in the SC + MF group, compared to administration ethanolic extract (p = 0.027).

Also, a statistically significant effect on the activity of GPx enzyme had both methanolic extract (p = 0.002) and ethanolic extract (p = 0.001) in the group most exposed to stress, SC + NMS + MF. At the same time, we found a significant effect on the enzymatic activity of the two extracts used when they were administered in the SC + NMS group (p <0.001) (Figure 3.43).

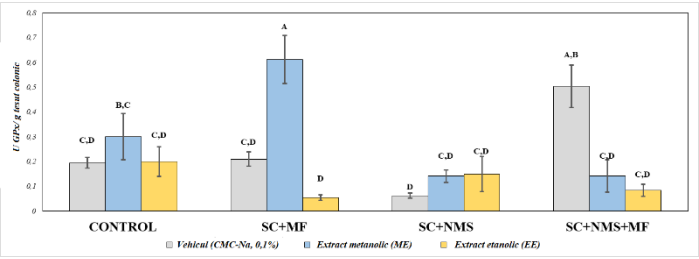


Figure 3.43. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on GPx activity at the colonic level in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B, C , D = Fisher's LSD test).

Lipid peroxidation at the colonic level experienced significant general variations after the administration of the two extracts (F 11, 96 = 9.66, p = 7.37E − 12). Moreover, it was observed that the ethanolic extract used in the group of non-stressed mice has the potential to induce a significant increase in MDA levels (p <0.001). In addition, there are significant differences in the modulatory effect in terms of lipid peroxidation, effects induced by the two extracts, and which differ from each other when administered to mice not exposed to stress (p <0.001). Similarly, both extracts show a pro-oxidant effect in intestinal tissue, in terms of MDA levels assessed in the SC + NMS group (pEE = 0.001) and SC + MF (pME = 0.005) (Figure 3.44).

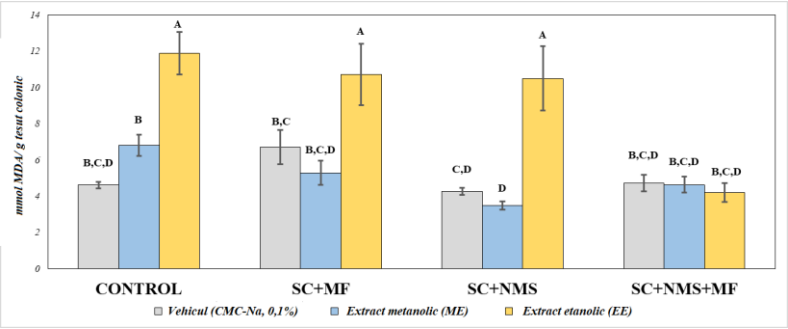


Figure 3.44. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on MDA levels in colonic tissue in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B, C , D = Fisher's LSD test).

At the same time, the highest yield obtained for the inhibition of lipid peroxidation was observed when the ethanolic extract was administered in the group most exposed to stress, SC + NMS + MF compared to all other groups that were treated with the same extract ($p < 0.001$) .

Total soluble proteins showed significant overall variations (F ð11, 96Þ = 7:80, p = 8: 03E − 10), and a statistically significant increased potential of methanolic extract to decrease the level of total soluble proteins in unstressed mice compared to the control group ($p = 0.02$). A similar effect was found in the groups SC + NMS ($p = 0.022$) and SC + NMS ($p = 0.015$), which received ethanolic extract compared to methanolic (Figure 3.45).

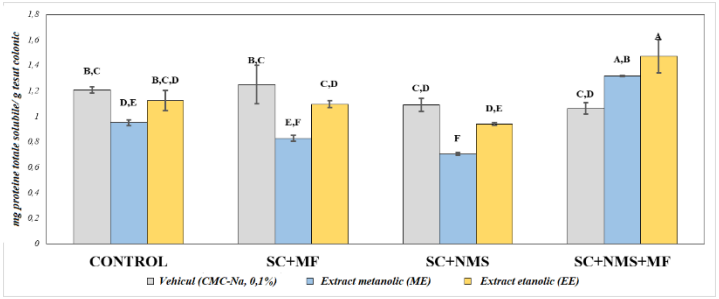


Figure 3.45. Effects of methanolic (ME) and ethanolic (EE) extracts obtained from the seeds of *Camelina sativa* var. Madalina on total soluble protein levels in brain tissue in an animal model of irritable bowel syndrome in mice. Values are expressed as mean ± S.E.M (n = 3 per group; C = control; SC = contention stress; NMS = neonatal maternal separation; MF = multifactorial stress; ME = methanolic extract; EE = ethanolic extract, A, B, C , D, E, F = Fisher's LSD test).

3.1.2.5. Evaluation and stained cytological examination of faeces

Following microscopic evaluation, slight variations in the observed elements could be identified. In the control group, yeasts are common or present in equal proportions. A similar frequency was observed in the other groups. As for erythrocytes, they have a lower frequency compared to yeasts in all four groups evaluated (present or rare). Inflammatory cells (lymphocytes) were found in a low frequency (rare and very rare). The presence of polymorphic flora can also be observed in most of the evaluated smears.

3.1.3. Results from the application of the multifactorial stress paradigm to rats and the administration of *Chrysanthellum americanum* extract

3.1.3.1 Evaluation of behavioral parameters

3.1.3.1.1. The effect of polyphenolic extract of *Chrysanthellum americanum* on short-term memory

Behavioral analysis using the Y-maze test revealed significant changes in the overall variation in the percentage of spontaneous alternations between groups of rats (F 3, 12 = 5.11, p = 0.009). Although the decrease in the percentage of spontaneous alternations in the group of rats with symptoms of irritable bowel syndrome (IBS group) compared to the control group was not statistically significant (F 1, 9 = 5.73, p = 0.31), the percentage mentioned was significantly lower compared to the group that received only extract (F 1, 9 = 5 73, p <0.034). Another statistically significant decrease among the studied groups was not identified (Figure 3.49).

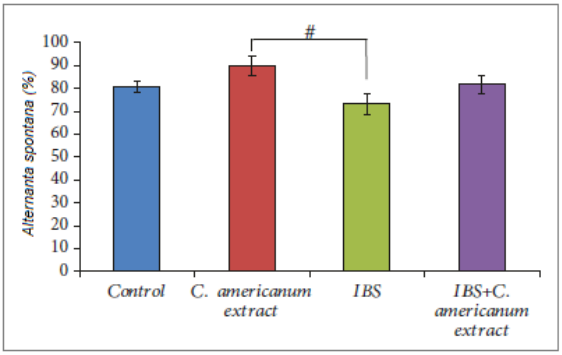


Figure 3.49. The effect of treatment with polyphenolic extract of *Chrysanthellum americanum* on short-term memory in an animal model of irritable bowel syndrome in rats in the Y-maze test, expressed as spontaneous alternation (%). Values are expressed as means ± S E M (n = 6 per group; #p = 0.03 for the group treated with C. americanum extract vs. IBS group).

3.1.3.1.2. The effect of polyphenolic extract of *Chrysanthellum americanum* on anxiety behavior

The evaluation of the anxious behavior using elevated plus maze test, following the administration of the polyphenolic extract of *Chrysanthellum americanum*, revealed that the plant shows anxiolytic action, by the high number of open arms entries that the rats registered in the IBS group and subsequently treated with extract versus IBS group (F 1.10 = 3.46, p = 0.09). The same high number of open arms entries was found in the control group, respectively the one who was given only the extract (Figure 3.50). The fact that there is no statistically significant difference can be explained by the general tendency of rats to make no more than one entry into the open arms, with the exception of the IBS group, which avoided exploration in the open arms.

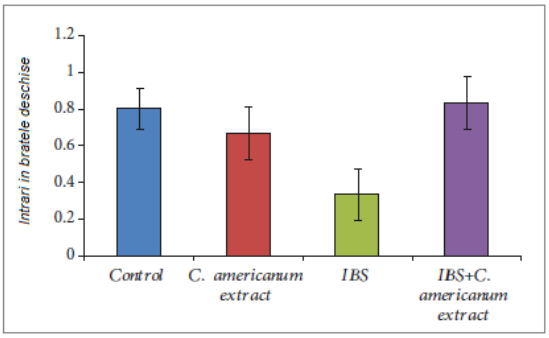


Figure 3.50. The effect of treatment with the polyphenolic extract of *Chrysanthellum americanum* on the anxious behavior in an animal model of irritable bowel syndrome in rats in elevated plus maze test, expressed as the number of open arms entries. Values are expressed as means ± S E M (n = 6 per group).

Moreover, these results were confirmed by analyzing the time spent in the open arms of the device (Figure 3.51). The ANOVA statistical test showed that treatment with polyphenolic extract of *Chrysanthellum americanum* significantly increased the time spent in the open arms in the group treated with extract compared to the IBS group ($F\ 1.10 = 5.18$, $p < 0.04$), and also in IBS group that received extract compared to IBS group ($F\ 1.10 = 5.21$, $p < 0.04$).

A statistically significant decrease in the open arms time of the IBS group compared to the control group ($F\ 1.10 = 8.81$, $p < 0.02$) also suggests an increased level of anxiety in the rats induced symptoms similar to irritable bowel syndrome.

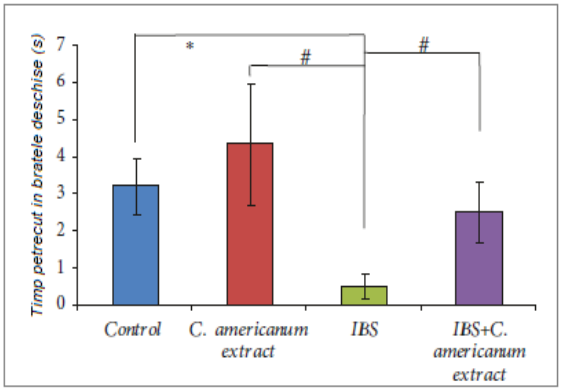


Figure 3.51. The effect of treatment with polyphenolic extract of *Chrysanthellum americanum* on anxious behavior in an animal model of irritable bowel syndrome in rats, in the elevated plus maze test, expressed as time spent in open arms. Values are expressed as means ± S E M (n = 6 per group ∗ p = 0.02 for the IBS group vs. the control group; #p = 0.04 for the group treated with extract, respectively the one with IBS and subsequently treated with *Chrysanthellum americanum* extract vs. the group with IBS).

3.1.3.1.3. The effect of polyphenolic extract of *Chrysanthellum americanum* on depressive behavior

The evaluation of the depressive behavior in experimental animals was conducted usig the forced swimming test which revealed antidepressant properties of the polyphenolic extract that we used. Statistically significant overall differences were observed between the groups studied, both in terms of swimming time ($F\ 3.20 = 5.86$, $p < 0.0048$) (Figure 3.53) and time spent in immobility ($F\ 3, 20 = 5.55$, $p < 0.0061$) (Figure 3.54).

While repeated exposure to stressors resulted in depression-like behavior in the IBS group, this was highlighted by significantly lower swimming time ($p = 0.01$) (Figure 3.53) and increased immobility ($p = 0.035$) (Figure 3.54).) both compared to the control group, the polyphenolic extract showed an antidepressant effect for both groups to which they were administered (extract group and IBS + extract group). The most important aspect, however, is this beneficial reversal of the effect of the extract that stands out in the IBS group and treated with extract, suggested by the significant

increase in swimming time (p = 0 03), compared to the IBS group (Figure 3.53) and the reduction of the immobility period (p = 0.031992), compared to the IBS group exposed to stress (Figure 3.54).

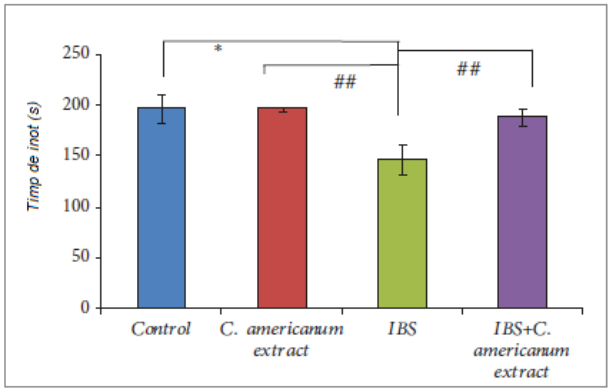


Figure 3.53. The effect of treatment with polyphenolic extract of *Chrysanthellum americanum* on depressive behavior in an animal model of irritable bowel syndrome in rats, usig forced swimming test, expressed as time spent swimming. Values are expressed as means ± S E M (n = 6 per group, ∗ p = 0.01 for IBS group vs. control group; ## p <0.01 for group treated with extract, respectively with IBS and subsequently treated with *Chrysanthellum americanum* vs IBS group).

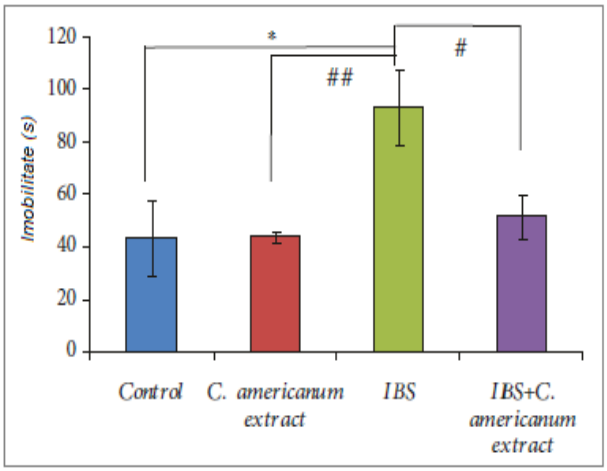


Figure 3.54. The effect of treatment with polyphenolic extract of *Chrysanthellum americanum* on depressive behavior in an animal model of irritable bowel syndrome in rats, usig forced swimming test, expressed as a period of immobility. Values are expressed as means ± S E M (n = 6 per group, ∗ p = 0.01 for the IBS group compared to the control group, #p ≤ 0.05 for the IBS group compared to the IBS group treated with *Chrysanthellum americanum* extract; ## p < 0 01for the group treated with *Chrysanthellum americanum* vs extract (IBS group).

3.1.3.2. Biochemical evaluation of oxidative stress biomarkers

After completion of the stress exposure protocol, GPx enzyme activity, as measured by the temporal lobe, showed a significant decrease in the IBS group compared to the control group (F 1, 11 = 12.93, p = 0.0042).

Similarly, a significant difference was observed between the extract-treated group and the IBS group (F 1, 11 = 15.69, p = 0.0022). No statistically significant differences were observed between the IBS group and the IBS group treated with extract; In any case, the activity of the enzyme in the latter group did not differ significantly from the activity evaluated in the non-stressed group, which suggests an improvement in the activity of the antioxidant that we used (Figure 3.55).

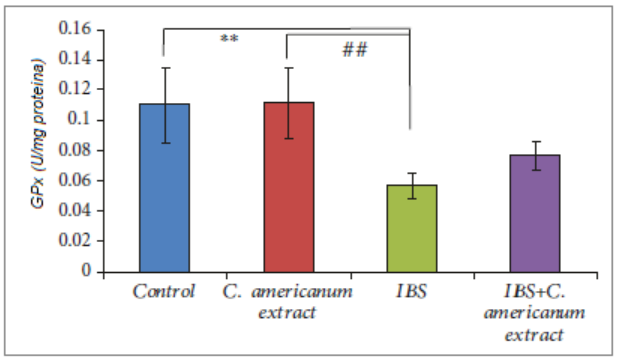


Figure 3.55. The effect of treatment with polyphenolic extract of *Chrysanthellum americanum*, after exposure to stress, on GPx activity in the temporal lobe. Values are expressed as means ± S E M (n = 6 for each group; total p = 0.001; ∗∗ p <0.01 for the IBS group vs. control group; ## p <0 01 for the group treated with *Chrysanthellum americanum* extract vs. IBS group).

The activity of the SOD enzyme in the brain tissue recorded significant general differences between the groups created in the researched experimental model (F 3, 25 = 6 83, p = 0 0016). Similar to GPx activity, there was a significant decrease in SOD activity in the IBS group, compared with both the control group (F 1.11 = 8.73, p = 0.013) and the group that received only polyphenolic extract of *Chrysanthellum americanum* (F 1 , 14 = 20.46, p = 0.00047) (Figure 3.56).

At the same time, the activity of the SOD enzyme decreased statistically significantly in the group exposed to stress and treated with extract compared to the control group on the one hand (F 1.11 = 5.34, p = 0.04), but also compared to the group that received only polyphenolic extract (F 1.13 = 10.44, p = 0.006) (Figure 3.56).

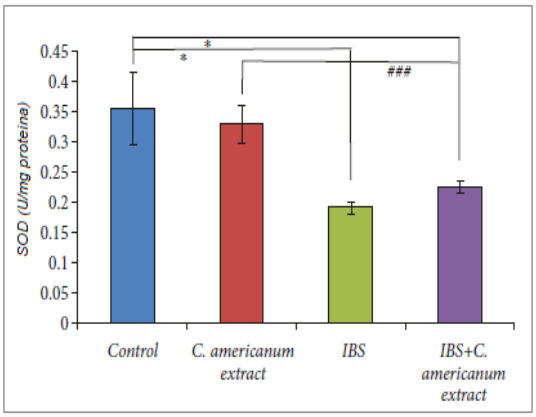


Figure 3.56. The effect of treatment with polyphenolic extract of *Chrysanthellum americanum*, after exposure to stress, on the activity of SOD in the temporal lobe. Values are expressed as means ± S E M (n = 6 for each group; total p = 0.001; ∗ p <0.05 for the IBS group and the IBS group subsequently treated with extract vs. control group; ### p <0.01 for the treated group with *Chrysanthellum americanum* extract vs. IBS group, respectively IBS group and subsequently treated with *Chrysanthellum americanum*).

The evaluation of one of the most important biomarker of lipid peroxidation, MDA, revealed a distinct and statistically significant increase in its levels in the stress-exposed group (IBS) compared to the other groups, as follows: p = 0.003 vs. control group, p = 0.011 vs. the group treated with extract and p = 0.014 vs. the treated group exposed to stress and treated with extract (Figure 3.57).

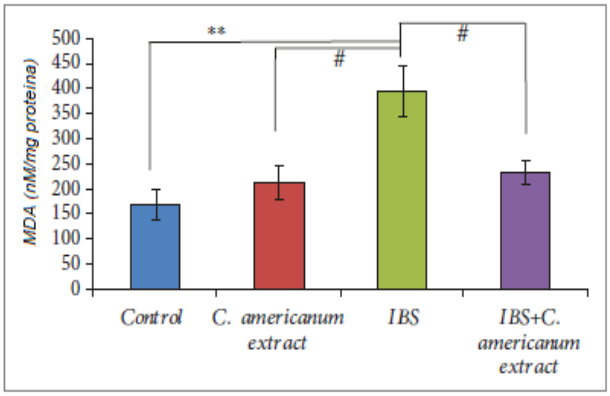


Figure 3.57. The effect of treatment with polyphenolic extract of *Chrysanthellum americanum*, after exposure to stress, on the level of MDA in the temporal lobe. Values are expressed as means ± S E M (n = 6 for each group; total p = 0.001; ∗∗ p <0.01 for the IBS group exposed to stress vs. the control group; #p <0.05 for the group treated with *Chrysanthellum americanum* extract , respectively the one with IBS and subsequently treated with extract vs. IBS group).

3.2. Results obtained from the study of human patients

3.2.1. Evaluation of the analog visual scale for irritable bowel syndrome (VAS-IBS)

The visual analogue scores for irritable bowel syndrome administered to participants were significantly higher in the group of patients with IBS compared to the control group of close age and same sex. The VAS-IBS results for each of the items are presented in Table 3.2. Thus, the intensity of physical symptoms such as abdominal pain (p <0.001), diarrhea (p = 0.021), constipation (p <0.001) or bloating / gas (p <0.001) significantly increased patients with IBS.

Table 3.2. Demographic and pathophysiological description of the study groups

	Study groups				p-value*
	Control (n = 14)	IBS (n = 10)			
ROME IV diagnostic criteria	-	Total n = 10	IBS – C n = 7	IBS – D n = 3	
Age (means ± SEM, years)	39,43 ± 4,801†	42,60 ± 6,46†	37,57 ± 8,04	54,33 ± 8,68	*†p=0.706*
Sex ratio (F / M %)	50% M/ 50% F	50% M/ 50% F	57,15% M/ 42,85% F	33,3% M/ 66,6% F	
Pittsburg Sleep Quality Index (PSQI)	1,786 ± 0,28	6,5 ± 1,118*	6,42 ± 1,601	6,66 ± 0,881	**p < 0.001*
Visual Analogue Scale for IBS (VAS-IBS)					
Abdominal pain intensity	1,428 ± 0,17	4,1 ± 0,65*	3,429 ± 0,68	5,67 ± 1,2	**p < 0.001*
Diarrhoea	1*	2,9 ± 0,91*	1,143 ± 0,142	7 ± 0,57	**p = 0.021*
Constipation	1,5 ± 0,35*	6,4 ± 0,93*	7,857 ± 0,34	3 ± 1,99	**p < 0.001*
Bloating/ gases	1,28 ± 0,12*	4 ± 0,76*	3,143 ± 0,50	6 ± 1,99	**p < 0.001*
Nausea/ vomiting	1*	1,8 ± 0,46*	1,714 ± 0,56	2 ± 0,99	**p = 0.053*
Perception of psychological wellbeing (emotional status)	8,64 ± 0,57*	4,3 ± 0,7*	4,429 ± 0,84	4 ± 1,52	**p < 0.001*
Daily life influenced GI problems (quality of life)	1,07 ± 0,07*	4,20 ± 0,48*	3,714 ± 0,60	5,333 ± 0,33	**p < 0.001*

**†Analysis of covariance*

While analyzing the results of VAS-IBS item scores by stratification in the IBS subtype, we observed that the frequency and intensity of abdominal pain were significantly higher in patients with the IBS-D subtype compared to patients with the IBS-C subtype (p <0.05) and age- and sex-appropriate healthy controls (p <0.001). In addition, symptoms of bloating and gas clearance were significantly more severe in patients with IBS-D subtype (p <0.01).

No significant differences were observed in terms of emotional state (item 4) and quality of life (item 5) between IBS subtypes (Figure 3.59), compared with healthy controls matched by age and sex ($p < 0.00$).

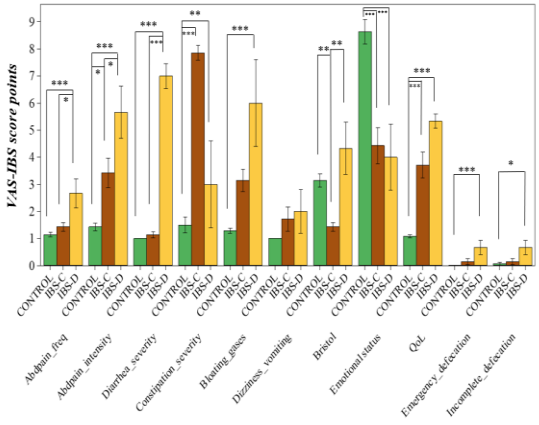


Figure 3.59. VAS - IBS scale parameter scores in IBS patients, stratified by IBS subtype. Results are expressed as mean VAS scale scores (ranging from 1 to 10, in direct correlation with severity) ± SEM (n = 14 for control, 10 for IBS, 7 for IBS-C and 3 for IBS-D, * $p < 0.05$, ** $p < 0.01$).

Comparing the results of VAS-IBS scale parameter scores by participant gender and diagnosis, we found that there were no significant differences between VAS-IBS item scores in men and women in the control group, while women with IBS experienced more frequent nausea and vomiting ($p = 0.065$), diarrhea ($p = 0.055$) and incomplete defecation ($p = 0.05$) (Figure 3.60). No significant differences were observed in terms of emotional state (point 4) and quality of life (point 5) between men and women.

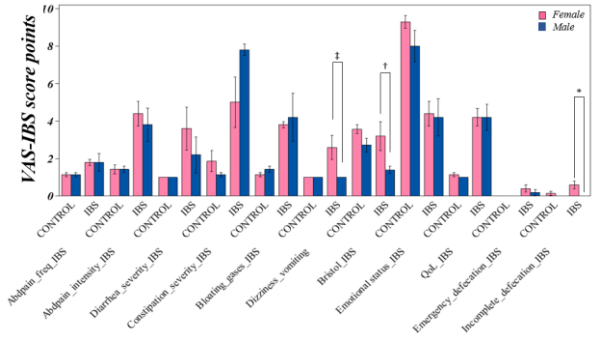


Figure 3.60. Scores of VAS-IBS scale parameters in patients with IBS and healthy volunteers, appropriate according to age and sex, stratified by sex. Results are expressed as mean VAS scale score (ranging from 1 to 10, in direct correlation with severity) ± SEM (n = 14 for control, 10 for IBS, 7 for IBS-C, and 3 for IBS-D, * $p < 0.05$, † $p = 0.055$, ‡ $p = 0.065$).

The differences between the groups compared for the parameters with numerical values and those with logical values indicate that, although the impairment is obvious, it is not perceived in a drastic way. However, these differences can be better observed by comparing the results obtained for IBS subtypes (Figure 3.61).

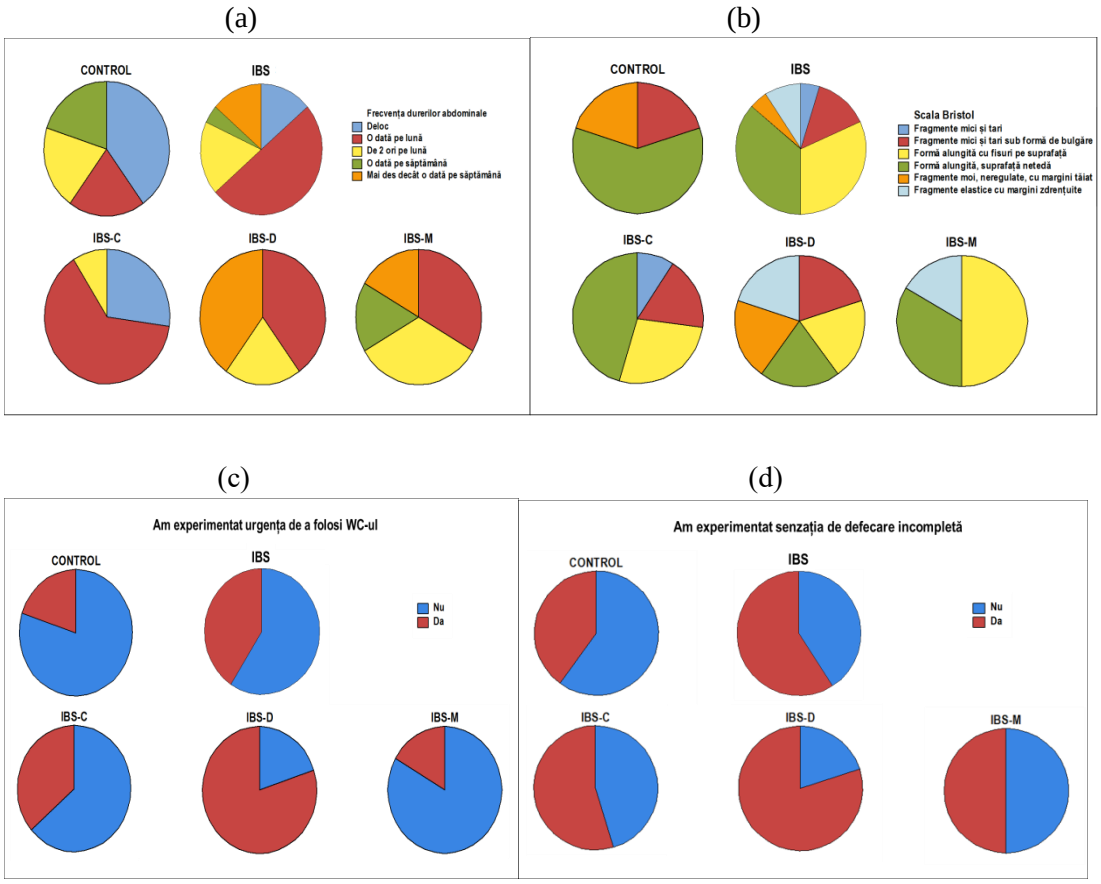


Figure 3.61. The variation of the parameters evaluated by the items with logical values of the VAS-IBS scale and the stratification of the results according to the type of IBS: (a) the frequency of abdominal pain; (b) Bristol scale; (c) urgency to defecate; (d) feeling of incomplete defecation.

3.2.2. Results of assessment of sleep disorders

For the Pittsburgh Sleep Quality Index (PSQI) questionnaire, no significant differences were observed in participants' age and gender. A PSQI rating of 0 to 5 suggests normal sleep quality, while an index greater than 5 certainly characterizes poor sleep.

In our study, we obtained a normal mean sleep quality index for the control group and increased PSQI for the IBS group, which is significantly different from the first group ($p < 0.001$) (Table 3.2). Stratification of the IBS subtype of the results revealed a significantly higher PSQI index in both IBS-C and IBS-D subgroups compared to the control group ($p < 0.001$). However, no significant difference was observed between PSQI indices when comparing IBS-C with IBS-D (Figure 3.62). The combined stratification of groups in terms of gender and IBS subtype showed significant differences between men and women according to IBS subtype (Figure 3.63).

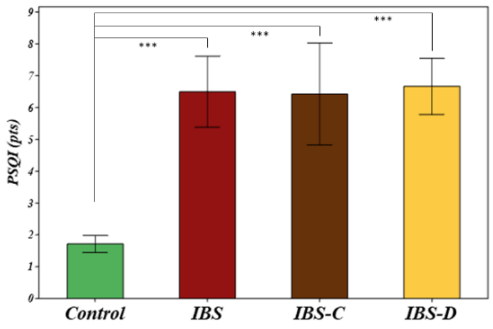


Figure 3.62. Sleep quality index assessed using the Pittsburgh Questionnaire (PSQI) in patients with IBS compared to healthy and same-sex volunteers. Results are expressed as mean PSQI ± SEM score (n = 14 for Control, 10 for IBS, *** p <0.001).

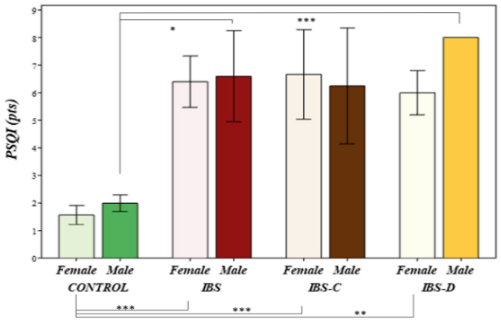


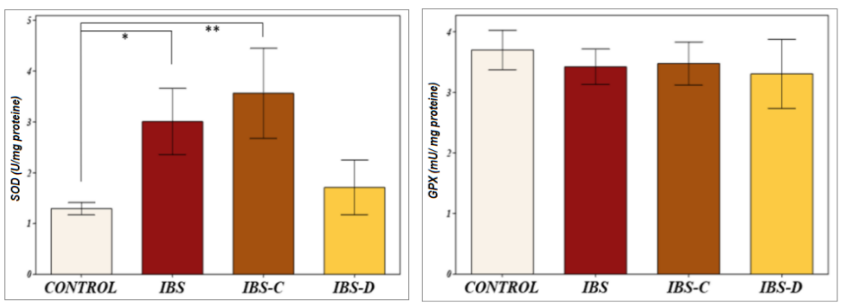
Figure 3.63. Sleep quality index assessed using the Pittsburgh Questionnaire (PSQI) in patients with IBS, stratified by patient gender and IBS subtypes. Results are expressed as mean ± SEM (n = 14 for control, 10 for IBS, 7 for IBS-C and 3 for IBS-D, * p <0.05, ** p <0.01, *** p <0.001).

Given the analysis of the items on sleeping habits (points 1-4), we observed that patients with IBS wake up considerably earlier compared to the control group (p = 0.022) and also spend more time trying to fall asleep (p = 0.026). Although there were no significant differences in bedtime, IBS patients reported that they slept, on average, much less time compared to those in the control group, appropriate in terms of age and sex (p = 0.02).

3.2.3. The results obtained from the biochemical analysis of the tears

Biochemical analyzes of the tear samples did not show significant differences between the two eyes of the participants. Also, no statistically significant differences were obtained between the samples of tears collected from women and men. Regarding the differences that appear between the two primary groups, we observed that in the IBS group, the SOD enzyme activity of the patients [F (1, 46) = 5.84, p = 0.020], the MDA levels (F 1, 46 = 7.85, p = 0.007), and total soluble protein levels (F1, 46 = 5.94, p = 0.019) recorded statistically significant increases compared to healthy study participants of the same sex and age (Figure 3.64).

There were no significant differences for GPx activity by comparing the group of IBS patients with the control group (F1, 46 = 0.36, p = 0.55). Analysis of the results after stratification by IBS subtype did not indicate any statistically significant differences in the levels of oxidative stress markers between the samples of patients with IBS-C and IBS-D. The analysis of the results by stratification according to gender and group of participants showed some differences that can be seen in Figure 3.65.



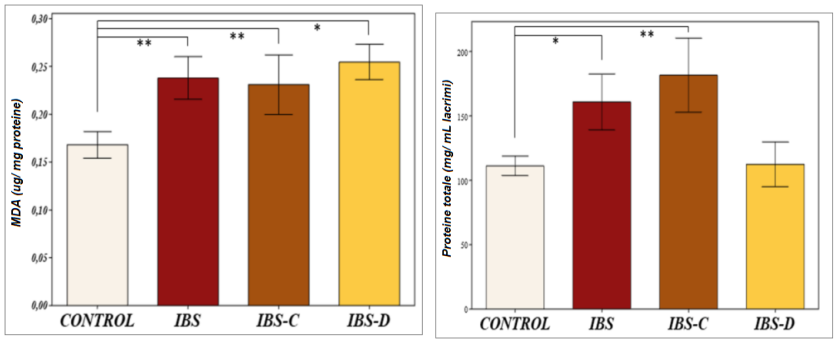


Figure 3.64. Markers of oxidative stress measured from tears in patients with IBS (IBS = all patients; IBS-C, IBS-D = groups of patients with stratified IBS by subtype) and healthy controls of the same age and sex: a. Superoxide dismutase activity (U / mg protein); b. Glutathione peroxidase activity (U / mg protein); c. Malondialdehyde concentration (µg MDA / mg protein); d) Total soluble protein (mg / ml tears). Results are expressed as means ± SEM (n = 14 for Control, 10 for IBS, 7 for IBS-C and 3 for IBS-D, * p <0.05, ** p <0.01).

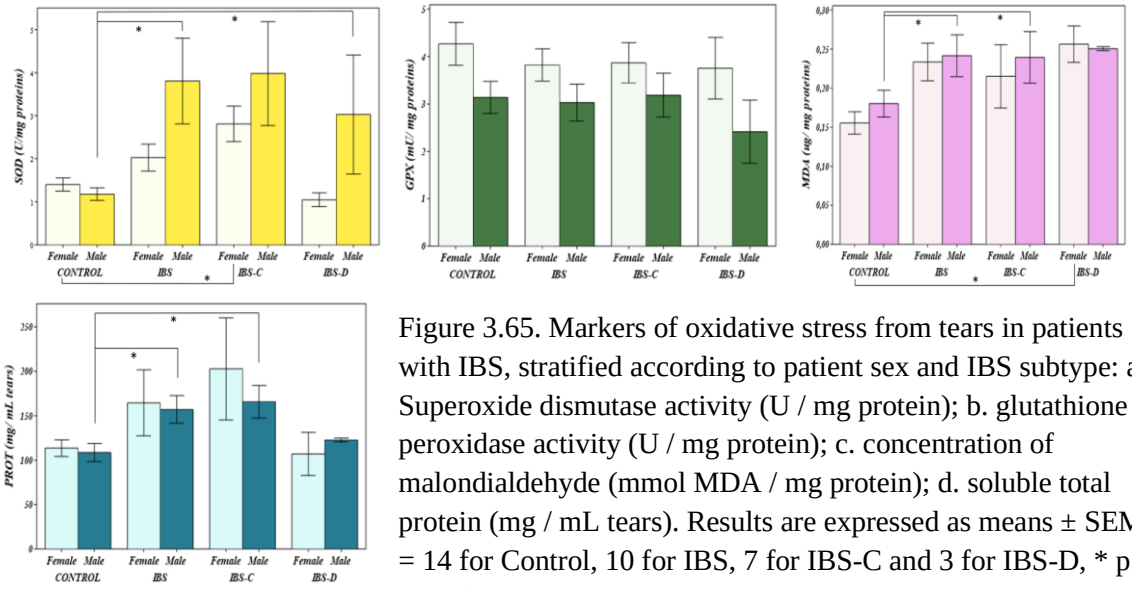


Figure 3.65. Markers of oxidative stress from tears in patients with IBS, stratified according to patient sex and IBS subtype: a. Superoxide dismutase activity (U / mg protein); b. glutathione peroxidase activity (U / mg protein); c. concentration of malondialdehyde (mmol MDA / mg protein); d. soluble total protein (mg / mL tears). Results are expressed as means ± SEM (n = 14 for Control, 10 for IBS, 7 for IBS-C and 3 for IBS-D, * p <0.05).

3.2.5. Results obtained from biochemical and immunological analyzes of serum, urine and faeces

The biochemical and immunological analysis of the relevant markers obtained from blood serum, urine and faeces, in the study of irritable bowel syndrome, showed variations depending on the biological product and the studied group. In serum, significant differences were observed for GPx enzymatic activity (p <0.001, by comparing IBS versus Control and IBS-D, IBS-C, IBS-M versus Control), but also for xanthine oxidase enzymatic activity (p <0.001). <0.05, by comparison IBS-D, IBS-C, IBS-M versus Control). At the same time, statistically significant variations were registered in the case of MDA concentration (p <0.05, by comparing IBS-D, IBS-C, IBS-M versus Control).

In urine, significant differences were observed for the enzymatic activity of GPx (p <0.05, by comparison of IBS versus Control), but also for the concentration of nitric oxide equivalents (µM nitrate) (p <0.05, by comparison of IBS versus Control).

Statistically significant differences were also observed in the faecal biochemical analysis for SOD ($p < 0.01$, by comparison of IBS-D, IBS-C, IBS-M versus Control), MDA ($p < 0.01$, by comparison of IBS-D, IBS- C, IBS-M versus Control) and nitric oxide ($p < 0.05$, by comparing IBS-D, IBS-C, IBS-M versus Control) (Figure 3.66).

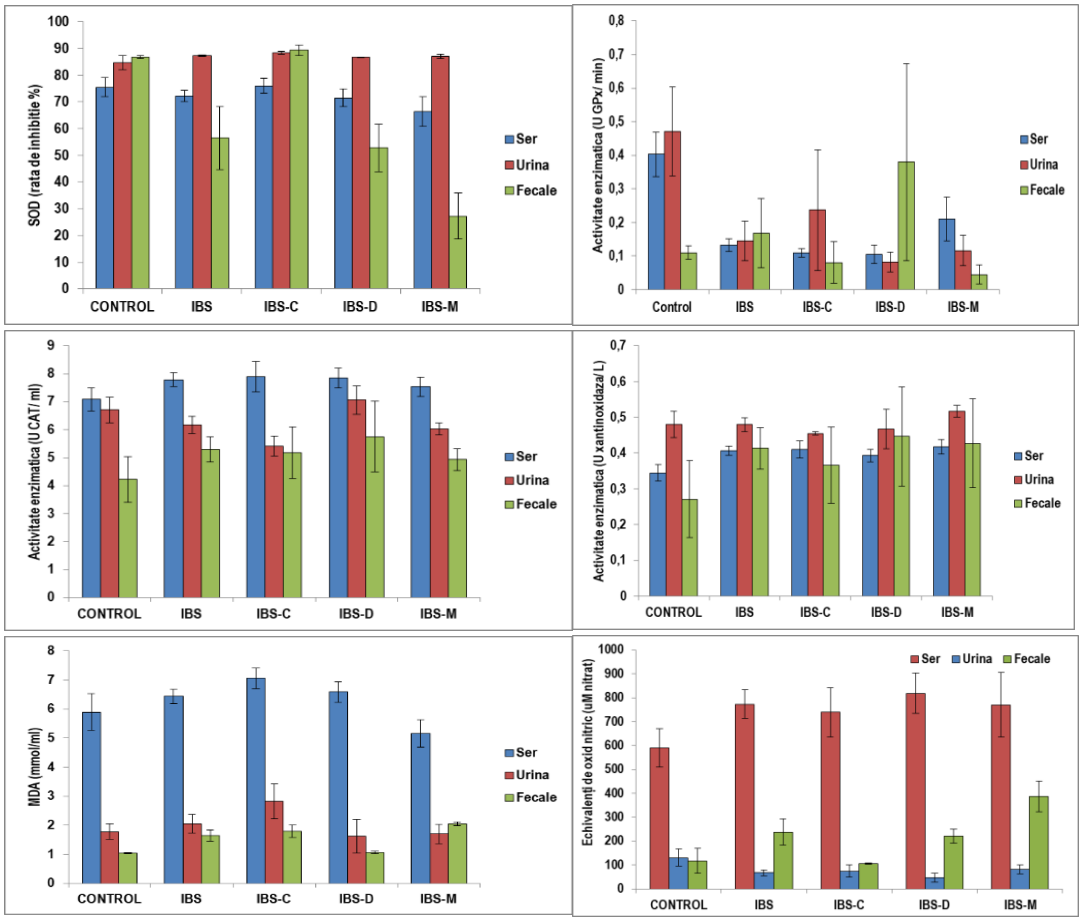


Figure 3.66. Variation of biochemical parameters by stratification of results according to the subtype of IBS, serum, urine and faeces: a. SOD activity, b. GPx activity, c. Catalase activity, d. Xanthine oxidase activity, e. MDA concentration, f. Equivalents of nitric oxide. Results are expressed as means ± SEM (n = 15 for Control, 15 for IBS-M, 15 for IBS-C and 15 for IBS-D)

Similarly, analysis of inflammatory and neuroendocrine parameters shows significant differences depending on the biological material used and the study group: serum for IL6 ($p < 0.05$, by comparing IBS versus Control and IBS-D versus IBS-C versus IBS-M) and IL8 ($p < 0.001$, by comparing IBS versus Control and IBS-D, IBS-C, IBS-M versus Control and comparing subtypes with each other), and of urine for serotonin ($p < 0.001$, by comparing IBS-D, IBS -C, IBS-M versus Control and comparing subtypes with each other) (Figure 3.67).

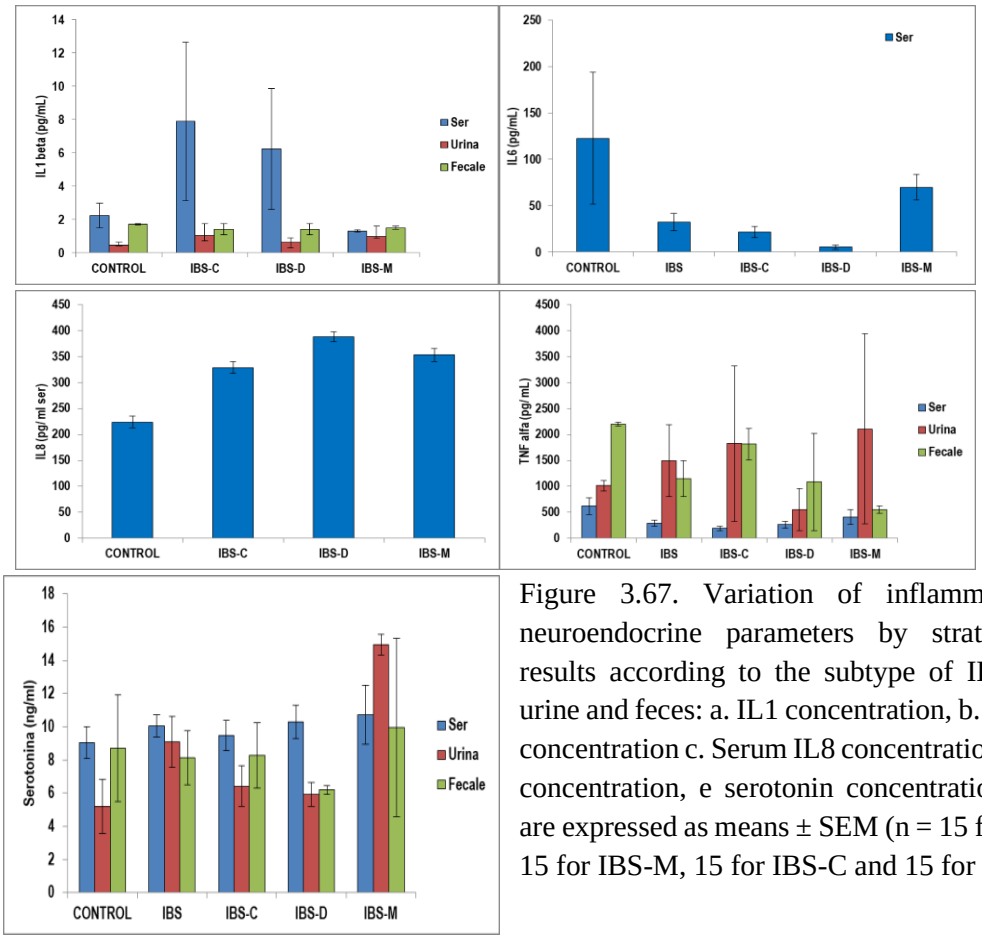


Figure 3.67. Variation of inflammatory and neuroendocrine parameters by stratifying the results according to the subtype of IBS, serum, urine and feces: a. IL1 concentration, b. Serum IL6 concentration c. Serum IL8 concentration, d. TNFα concentration, e serotonin concentration. Results are expressed as means ± SEM (n = 15 for Control, 15 for IBS-M, 15 for IBS-C and 15 for IBS-D)

The evaluation of the data according to the sex of the study participants, in the context of the subtypes of irritable bowel syndrome, did not reveal statistically significant variations for the biochemical parameters (Figure 3.68). In contrast, there were some obvious increases in IL1 levels in the IBS-C group, serotonin in the IBS-M group, and TNFα in the IBS-M group, respectively in men compared to women in the same group (Figure 3.69).

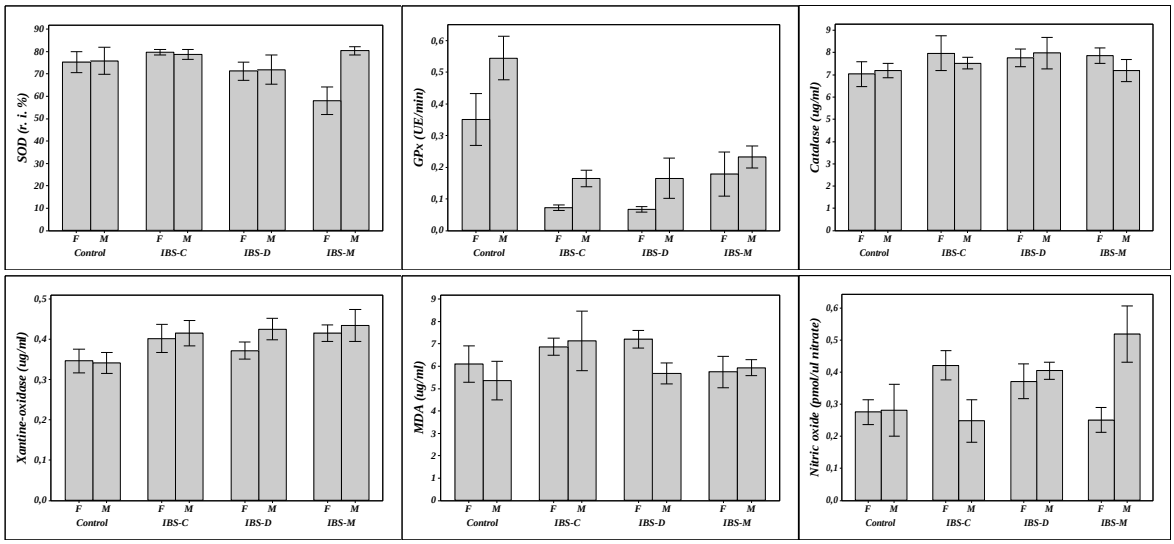


Figure 3.68. Variation of serum biochemical parameters by stratification of results according to the subtype of IBS and sex: a. SOD activity, b. GPx activity, c. Catalase activity, d. Xanthine oxidase activity, e. MDA concentration, f. Nitric oxide equivalents. Results are expressed as means ± SEM (n = 15 for Control, 15 for IBS-M, 15 for IBS-C and 15 for IBS-D)

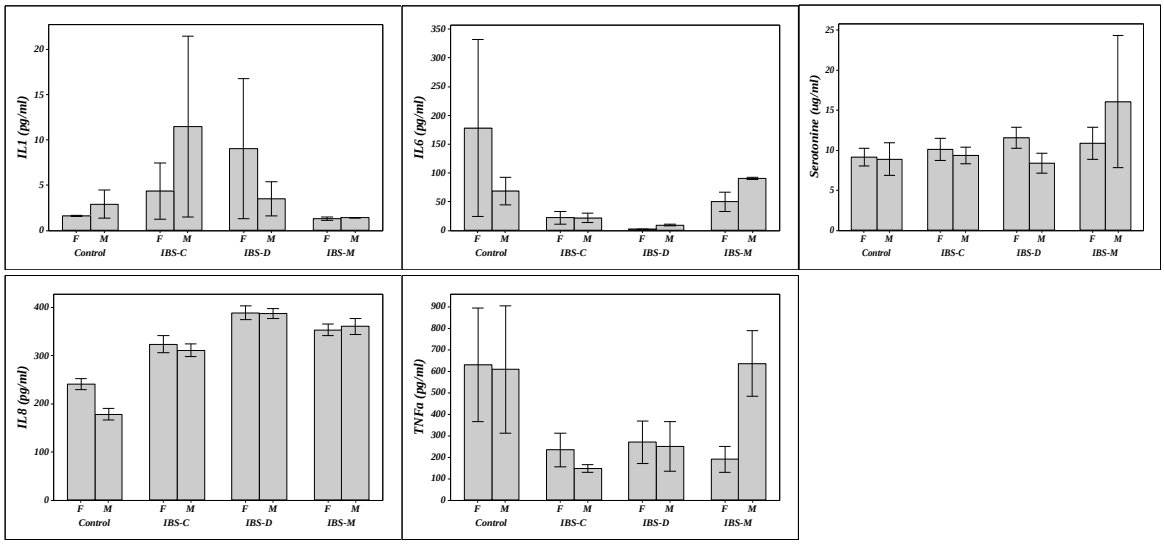


Figure 3.69. Variation of inflammatory and neuroendocrine serum parameters by stratification of results according to IBS subtype and sex: a. IL1 concentration, b. Serum IL6 concentration c. Serum IL8 concentration, d. TNFα concentration, e. Serotonin concentration . Results are expressed as means ± SEM (n = 15 for Control, 15 for IBS-M, 15 for IBS-C and 15 for IBS-D).

GENERAL CONCLUSIONS

Following the experiments that are the subject of this thesis, the conclusions deduced in this point of the research are the following:

1. Irritable bowel syndrome is a gastrointestinal condition that is becoming more common in clinical practice with a poorly known and understood etiopathogenesis, most likely multifactorial, with a number of interconnected mechanisms that are closely related to the onset of this syndrome.

2. The multifactorial nature highlights the need to evaluate this syndrome from different perspectives, both through an approach to behavioral manifestations and biochemical and immunological biomarkers.

3. The study, following three distinct directions, provides evidence on the link between irritable bowel syndrome, cognitive and psycho-emotional impairments, and changes in oxidative status in both experimental animal models and human patients.

4. The animal model of irritable bowel syndrome by exposing mice to multifactorial stress provides further evidence on the effect of stress exposure on the gastrointestinal tract and neurological status. The combination of exposure to neonatal maternal separation stress and chronic stress, encountered in adulthood, can lead to depressive and anxiety behaviors, accompanied by changes in intestinal transit.

5. Exposure to various stressors could give rise to a central response with an immediate effect compared to that caused by early life stress, but in general the combination of the two types of stressors indicated more accurately visceral and irritable bowel syndrome-specific symptoms, arguing for the need for a higher-precision IBS model in mice.

6. Study on the therapeutic effects of methanolic and ethanolic extracts from the seeds of *Camelina sativa* var. Madalina indicated a beneficial direction on behavioral issues and oxidative status in the brain and colon in mice, in a model of irritable bowel syndrome based on exposure to stress.

7. Despite the small differences in the chemical composition of the methanolic and ethanolic extracts, the results obtained suggested that the extracts obtained from the seeds of the plant *Camelina sativa* var. Madalina could reverse the short-term memory disorders caused by exposure to stress. At the same time, the intensity and frequency of anxiety and depression-like behaviors observed in stress-induced animal models of irritable bowel syndrome may decrease.

8. Extracts obtained from the seeds of *Camelina sativa* var. Madalina showed a significant effect on markers of oxidative stress in the brain and intestinal tissues in mice exposed to the paradigm of multifactorial stress, by decreasing the activity of superoxide dismutase and increasing the activity of glutathione peroxidase. However, the results suggest that the extracts may also increase lipid peroxidation in intestinal tissues.

9. Following microscopic evaluation of faeces, the most consistent elements identified are yeasts (common in the control group), in all groups evaluated (present or rare). Red blood cells had a low frequency, as did inflammatory cells (lymphocytes) (rare and very rare). The polymorphic flora was also found in most of the evaluated smears. Therefore, slight variations in the observed but insignificant elements could be identified.

10. This study provides further evidence that the administration of alcoholic extracts from the seeds of *Camelina sativa* var. Madalina could improve cognitive performance and mood, showing antioxidant capacity in both the brain and intestinal tissues.

11. The study, which is based on the polyphenolic extract of *Chrysanthellum americanum* used in a stress-induced animal model of irritable bowel syndrome, also indicated its influence on oxidative status on the one hand, and a slight improvement in memory along with a significant decrease in anxiety and depression-like behavior.

12. Evaluations of markers of oxidative stress indicated a significant decrease in the activity of superoxide dismutase and glutathione peroxidase in the temporal lobes in rats involved in the animal model of irritable bowel syndrome obtained by exposure to stress. Short-term administration of polyphenolic extract significantly attenuated elevated malondialdehyde levels and specific glutathione peroxidase activity. Significant Pearson correlations were also established between oxidative stress biomarkers and behavioral indicators.

13. Administration of *Chrysanthellum americanum* polyphenolic extract can alleviate the mood and cognitive impairment associated with stress-induced irritable bowel syndrome by improving oxidative stress in the brain.

14. The final form of the Romanian VAS-IBS scale can be considered a reliable tool in measuring the perceived severity of gastrointestinal symptoms, which can be classified by ROME IV as symptoms of irritable bowel syndrome.

15. The study involving human patients provided further evidence of the involvement of oxidative stress in the pathology of irritable bowel syndrome. Therefore, we identified significant changes in the activity of superoxide dismutase, malondialdehyde in the tear samples of patients with IBS, compared to healthy volunteers participating in the study. However, no significant differences were observed for glutathione peroxidase activity.

16. The altered oxidative status in the tear samples was not correlated with sleep disorders that occur in patients with IBS, except for sleep disorders that occur due to painful events, but was significantly correlated with gastrointestinal symptoms.

17. Assessment of sleep quality using the Pittsburgh Questionnaire (PSQI) did not reveal any significant differences in participants' age and sex, with a normal average sleep quality index for healthy volunteers and a statistically significant decrease in sleep quality in IBS group. Also, although there was a significant difference between the IBS-C and IBS-D subgroups compared to

the control group, no difference was observed between the PSQI indices when the two IBS subgroups were compared.

18. Patients with IBS wake up much earlier than healthy volunteers and spend more time trying to fall asleep, sleeping, on average, less than participants in the control group. The identification of sleep disorders in patients with IBS by evaluating the PSQI questionnaires provides additional evidence related to the causal relationship between the disorders and IBS symptoms.

19. There are significant differences between men and women in the dynamics of oxidative stress markers, that women, although more likely to develop more aggressive symptoms of the syndrome, experience improvements in oxidative status due to better antioxidant defense.

Personal contributions: conducting most experiments on animal behavior, performing biochemical and immunological determinations of biological products (serum, tears, urine, faeces) obtained from healthy patients and volunteers as well as those obtained from experimental animals, performing and evaluating smears obtained from the faeces of the experimental animals (coprocytograms), completion of the PSQI questionnaires and the VAS-IBS scale, production and statistical interpretation of the data obtained (partial participation).

Limitations of the study: Regarding the limitations of our studies, we must mention that the animal model used is based on a previously validated animal model, previous protocols, which we have adapted to obtain the effect that stress causes. Another limitation of the study is the small number of animals tested and the absence of drug treatments for a positive control group. In the case of animal models, the results should be treated with care and caution, as they reproduce only some aspects of the pathology, so it is necessary to evaluate them as the first steps in observing the phenomena characteristic of irritable bowel syndrome in the context of a wide pathology. psychiatrist.

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LIST OF OWN WORKS

I. ISI published articles

1. **Roxana Cojocariu**, Alin Ciobica , Ioana-Miruna Balmus, Samson Guenne, Anca Trifan , Carol Stanciu, LuminitaHrițcu, and Radu Lefter, 2019, Antioxidant Capacity and Behavioral Relevance of a Polyphenolic Extract of Chrysanthellum americanum in a Rat Model of Irritable Bowel Syndrome, Hindawi Oxidative Medicine andCellularLongevity Volume 2019, Article ID 3492767, 13 pageshttps://doi.org/10.1155/2019/3492767).

2. **Roxana O Cojocariu**, Ioana-MirunaBalmus, Radu Lefter, Luminita Hritcu, Daniela C Ababei, Alin Ciobica, Simona Copaci, Silvia E L Mot, Lucian Copolovici, Dana M Copolovici, Stefana Jurcoane, *"Camelina sativa* Methanolic and Ethanolic Extract Potential in Alleviating Oxidative Stress, Memory Deficits, and Affective Impairments in Stress Exposure-Based Irritable Bowel Syndrome Mouse Models", Oxidative Medicine and Cellular Longevity,2020 Dec 23; 2020:9510305.doi:10.1155/2020/9510305. eCollection.

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4. 4.Ioana-Miruna Balmus, **Roxana-Oana Cojocariu**, Alin Ciobica, Stefan Strungaru, Roxana Strungaru-Jijie, Alina Cantemir, Catalina Galatanu, Lucian Gorgan "Preliminary Study on the Tears Oxidative Stress Status and Sleep Disturbances in Irritable Bowel Syndrome Patients" Oxidative Medicine and Cellular Longevity, 23 mai 2020; doi:10.1155/2020/4690713. E Collection 2020.

5. Radu Lefter, **Roxana Oana Cojocariu**, Alin Ciobica, Ioana-Miruna Balmus, Ioannis Mavroudis, Anna Kis, "Interactions between Sleep and Emotions in Humans and Animal Models"Medicina (Kaunas), 2022 Feb 11;58(2):274. doi: 10.3390/medicina58020274.

6. **Roxana Cojocariu**, Ioana Balmus, Radu Lefter, Luminita Hritcu, Dana Ababei, Alin Ciobica, Simona Copaci, Lucian Copolovici, Dana Copolovici, StefanaJurcoane, „Beneficial effects of *Camelina sativa* oil on behavioural (memory, anxiety, depression and social-related) manifestations and oxidative stress parameters in a

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8. Bogdan Doroftei, Ovidiu-Dumitru Ilie, **Roxana-Oana Cojocariu,** Alin Ciobica, Radu Maftei, Delia Grab, Emil Anton, Jack McKenna, Nitasha Dhunna and Gabriela Simionescu "Minireview Exploring the Biological Cycle of VitaminB3 and Its Influence on Oxidative Stress: Further Molecular and Clinical Aspects" Molecules, iulie 2020, 22;25(15):3323. doi: 10.3390/molecules25153323.

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II. BDI published articles

1. **Roxana Oana Cojocariu**, Alin Ciobica, Daniel Timofte, 2018, „The current state of knowledge on the link between human microbiome and some neuropsychiatric disorders", Bulletin of Integrative Psychiatry, Nr. 2 (77).

2. **Roxana Oana Cojocariu,** Alin Ciobica, Daniel Timofte, 2018, „The relevance of somenewbiomarkers (e.g. oxidative stress) in psychiatry as evidencedby non-invasive methods in saliva, tears, urine or feaces", Bulletin of Integrative Psychiatry, Nr. 2 (77).

3. **Roxana Oana Cojocariu,** Alin Ciobica, Radu Lefter, Heba El- Lethey, Dumitru Cojocaru, Daniel Timofte, 2018, „Current Biomarkers in Irritable Bowel Syndrome", Academy of Romanian Scientists Annals Series on Biological Sciences, Volume 7, No. 2, pp. 27 - 47 ISSN 2285 – 4177.

4. **Roxana Oana Cojocariu**, Mihaela Gontea, Irina Dobrin, Daniel Timofte, 2018, „The possible metabolic and psychiatric relevance for a comparative analysis of transaminases in patients with well and poorly controlled type 2 diabetes", Bulletin of Integrative Psychiatry, Nr. 4 (79).

5. **Roxana Oana Cojocariu**, Alin Ciobica, 2019, „General Lines to Be Followed in the Ethics of Animal Models Studies for the Neuropsychiatric Disorders and Associated Manifestations", Academy of Romanian Scientists Annals Series on Biological Sciences Volume 8, No. 1, pp. 45 - 52 ISSN 2285 – 4177

6. **Roxana Oana Cojocariu**, Ioana Miruna Balmus, Radu Lefter, Alin Ciobica „Further Studies on the Neurological Component of Irritable Bowel Syndrome. The Connections Between Parkinson's Disease Pathology and Irritable Bowel Syndrome Manifestations") Annals Series on BIOLOGICAL SCIENCES volume 8 2019 Number 2 ISSN 2285 – 416

7. Ioana-MirunaBalmus, **Roxana Cojocariu**, Alin Ciobîcă, Alina Cantemir, CătalinaGălățanu, Carol Stanciu, Anca Trifan, Lucian Gorgan, Daniel Timofte „ Validation of romanian version of visual analogue scale for irritable bowel syndrome questionnaire (VAS-IBS)", Bulletin of Integrative Psychiatry, March 2020, DOI: 10.36219/BPI.2020.1.08

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III. Participation in international scientific events

1. **Roxana Oana Cojocariu**, Radu Lefter, Daniela Ababei, Alin Ciobica, prezentare orala cu titlul „The relevance of cognitive deficit in an adapted animal model of IBS in mice", 27 th European Congress of Psychiatry, Varsovia, Polonia, 6-9 aprilie, 2019

2. **Roxana Oana Cojocariu**, Miruna Balmus, Alin Ciobica, Radu Lefter, Daniela Ababei, Luminita Diana Hritcu, poster cu titlul „A synthesis on behavioural disorders in irritable bowel syndrome", 48th Meeting of the European Brain and Behaviour Society, Praga, 21-24 septembrie 2019

3. Miruna Balmus, **Roxana Oana Cojocariu**, Alin Ciobica, Radu Lefter, Daniela Ababei, Luminita Diana Hritcu, poster cu titlul „Depressive and anxious behaviours evaluation in adapted mice model of irritable bowel syndrome", 48th Meeting of the European Brain and Behaviour Society, Praga, 21-24 septembrie 2019.

IV. Participation in national scientific events

1. **Roxana Oana Cojocariu,** Alin Ciobica, poster cu titlul "O sinteză privind posibila legătura dintre boala Alzheimer și sindromul colonului iritabil, Cea de IX-a ediție a Conferinței Naționale Alzheimer cu participare internațională, București, 20 – 23 februarie 2019.

2. **Roxana Oana Cojocariu,** Alin Ciobica, Daniela Ababei, Miruna Balmus, Radu Lefter, prezentare orala cu titlul „Preliminary Data on Some Facilitatory Effects of Camelina Extracts on Two Different Mice Model of Irritable Bowel Syndrome on Mice", Conferinta Stiintifica a Academiei Oamenilor de Stiinta din Romania, Bucuresti, 4-6 aprilie 2019

3. **Roxana Oana Cojocariu**, Alin Ciobica , Ioana-Miruna Balmus, Samson Guenne, Anca Trifan, Carol Stanciu, Luminita Hrițcu, and Radu Lefter, prezentare orala cu titlul „Antioxidant Capacity and Behavioral Relevance of a Polyphenolic Extract of Chrysanthellum americanum in a Rat Model of Irritable Bowel Syndrome", Zilele medicamentului, Iasi, 10-12 octombrie 2019.

4. **Roxana Oana Cojocariu,** Miruna Balmus, Alin Ciobica, Radu Lefter, Daniela

Ababei, Luminita Diana Hritcu, prezentare orala cu titlul „Date preliminare privind eficiența separării neomaterne, stresul de contenție și expunere cronică la o combinație de factori stresori ușori și impredictibili într-un model complex de sindrom de colon iritabil la șoareci - evaluarea stresului oxidativ Conferinta Nationala Ştiintifica Academia Oamenilor De Ştiinta din Romania, Brasov, 20 - 21 septembrie 2019

5. **Roxana Oana Cojocariu,** Alin Ciobica, Ioana – Miruna Balmus, Lucian Gorgan, Manuela Padurariu, Carol Stanciu and Anca Trifan, 2019, EHB, „Some mechanistical and computational aspects on the correlations that might exist between irritable bowel syndrome versus sleep patterns and disturbances" EHB 2019 paper 47.pdf

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7. Ioana-Miruna Balmus, Alin Ciobica, **Roxana Oana Cojocariu**, Lucian Gorgan, Carol Stanciu and Anca Trifan, 2019, EHB," The Possible Biorelevance of Alcohol Consumption in some Gastrointestinal IBS-LikeSymptoms - Correlative Studies Based on Surveying" EHB_2019_paper_44.pdf 47

8. **Roxana Cojocariu,** Ioana-Miruna Balmuș, Radu Lefter, Daniela Ababei, Alin Ciobîcă, Luminița Hrițcu, prezentare orală cu titlul „Studierea deficiențelor cognitive într-un model animal pentru sindromul colonului iritabil obținut prin expunerea la stres multifactorial", Conferința Națională a Școlilor Doctorale din Consorțiul Universitaria, Ediția a II-a, 11 noiembrie - 14 noiembrie 2019, Timișoara.

9. **Roxana Oana Cojocariu**, Ioana Miruna Balmuș, Radu Lefter, Alin Ciobica, prezentare orală cu titlul „General Aspects to Be Followed in the Ethics of Animal Models Studies for the Neuropsychiatric Disorders", Conferința cu participare internațională Etica Cercetării și Publicării Științifice, UMF Iași, 4-5 decembrie 2019.