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STUDIES ON THE LINK BETWEEN ALZHEIMER'S DISEASE AND DIABETES

(IS ALZHEIMER'S DISEASE TYPE 3 DIABETES?)

SUMMARY

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LIST OF ABBREVIATIONS

Aβ - amyloid β peptide

ADDL – beta-amyloid-derived diffusible ligands

APP - amyloid precursor protein

AD - Alzheimer's disease

DSDS – right-left-right-left alternating tetragrams

GABA - gamma-aminobutyric acid

IL-1β – interleukin 1β

IL-6 – interleukin 6

MMSE - Minimum Mental Status Examination

NFT - neurofibrillary tangles

PSEN1 - presenilin-1

PSEN2 - presenilin-2

SDSD – left-right-left-right alternating tetragrams

CNS – central nervous system

TNF – tumor necrosis factor

YKL-40 – chitinase 3-like protein 1

INTRODUCTION

The current study brings to attention a pathology from clinical practice, which is increasingly common, namely, Alzheimer's disease, highlighted by the numerous publications, which have as their central subject the interest in the group of neurological disorders. This is a progressive neurodegenerative condition, the densest form of senile dementia, correlated with the aging process, characterized by the progressive decline of cognitive functions (thinking, memory, speech), behavioral and personality changes.

Although the cognitive decline, encountered predilection in the elderly, is considered to be a normal part of the process of change of an organism, which has passed the period of maturity, that is, of senescence, the existing publications demonstrate that this disorder also occurs among older people. young man Let's remember the fact that Alzheimer's disease was detected in a 50-year-old woman. Being under the observation of Alois Alzheimer, hence the name of this disorder, the patient displayed a series of symptoms, surprising for that age and comparable to other elderly patients diagnosed with dementia. Among the manifestations are behavioral and mood disorders, confusional states, persistent memory impairment up to the impossibility of performing usual daily activities. Over time, the total loss of autonomy is reached. All these manifestations are preceded by aphasia, agnosia and apraxia. There is research that claims that Alzheimer's disease would be based on metabolic disorders, vascular deficiencies, which, if diagnosed in time, and accompanied by various prevention strategies, for the initial stages of cognitive decline, the development of Alzheimer's disease could be prevented. These arguments could explain the presence of these symptoms in younger people as well.

One of the reasons to choose this research topic is the fact that Alzheimer's disease still represents a challenge, both in terms of diagnosis and treatment. Other motivating factors in choosing the research topic are the global impact on a large number of people, the estimated increase in their number in the near future, as well as the huge impact on medical resources, the estimated cost of medical care, and last but not least, the decrease the quality of daily life of individuals affected by this disease. We also want to understand the hypothesis that Alzheimer's disease is a form of diabetes that selectively affects the brain, by correlating the degree of cognitive degradation with the severity of diabetes, in patients diagnosed by specialist psychiatrists from the Socola Psychiatry Institute, Iasi, Romania.

As we stated before, among the priorities of today's society is the improvement of the quality of life of patients diagnosed with Alzheimer's disease. As a result, the pathogenesis of Alzheimer's disease must be studied in all its aspects, with the aim of discovering new therapeutic strategies in this regard, which is possible only through the existence of animal models.

Thus, the main purpose of this work is the generation of animal models by administration of an insecticide and sucrose and the behavioral assessement of their memory capacity, their exploratory tendencies, their aggressiveness, their social preference and anxiety, using suitable tests to highlight the existence of cognitive impairments, found among the symptoms of Alzheimer's disease. Basically, the purpose of these tests is to analyze the interactions between cognitive and metabolic deficiencies, different behavioral aspects, in animal models, aspects determined by various methods. From a neurological perspective, we wanted to find out if there is an overlap between the metabolic syndrome and neuropsychiatric disorders.

The element of novelty and originality of this research consists precisely in the generation of a combined animal model of Alzheimer's and diabetes, relevant for the main neurological pathologies in today's society.

The motivation for choosing this theme lies in understanding the hypothesis that Alzheimer's disease represents a form of diabetes specific to the brain. This represents yet another challenge of diagnosis and treatment, as well as predicting an increase in the number of people affected by this disease to one billion of the global population by 2030.

The objectives proposed for the animal models study include:

1. the generation of memory deficiencies similar to Alzheimer's disease in fish by administration of fipronil in combination with sucrose;
2. obtaining metabolic deficiencies specific to diabetes based on the administration of sucrose in different concentrations;
3. establishing interactions between metabolic deficiencies and those of a neurological nature within the generated animal model.

Regarding human patients, that make the object of the second part of this study, we aimed to analyse:

1. the correlation between the degree of cognitive degradation and the severity of diabetes;
2. studying how the specific medication for Alzheimer's disease and diabetes influences the pathological picture of these diseases and the interactions between them.

CHAPTER 1. THE CURRENT STATE OF RESEARCH ON ALZHEIMER'S DISEASE AND DIABETES

Alzheimer's disease is a progressive neurodegenerative condition, the densest form of senile dementia, correlated with the aging process, characterized by the progressive decline of cognitive functions (thinking, memory, speech), behavioral and personality changes. Alzheimer's disease is also called type 3 diabetes, a term proposed for this neurological disease, which results from insulin resistance of the brain; a type of diabetes specific to the brain.

Alzheimer's disease (named after the German psychiatrist Alois Alzheimer) is the most well-known type of dementia and can be defined as a slowly progressive neurodegenerative disease characterized by neuritic plaques and neurofibrillary tangles as a result of the accumulation of beta-amyloid peptide in the most affected area of the brain, medial temporal lobe and neocortical structures *(De-Paula V.J., 2012).*

Dementia is a general term used to describe a group of symptoms that affect a person's cognitive abilities, including memory, language, perception and problem-solving skills. It is not a specific disease, but rather a collection of symptoms that can be caused by different underlying conditions. This does not take into account ethnicity, social category, gender, age, so it can be called a "democratic" condition. It can be diagnosed using the Reisberg scale, the Mini Mental Examination Scale and the Clock Test *(Omer I. et al., 2014).*

Dementia symptoms can vary from person to person, but common signs include memory loss, language and communication difficulties, impaired judgment, confusion, personality changes, and difficulty performing daily tasks.

Alzheimer's disease is one of the most common forms of dementia. It is responsible for 75% of cases *(Qi C. et al., 2009)* on its own. Most of the time, however, it is associated with other forms of pathology, a situation in which it is called mixed dementia. It was first described over 100 years ago by the German psychiatrist Alois Alzheimer and is named after him *(Maurer K. et al., 1997).* At the onset of the disease, the primary characteristics would be the inability to remember recent events and difficulty finding words *(Taylor J-P., Thomas A., 2013).* As the disease progresses, there is greater memory loss as well as language difficulties, which become increasingly noticeable, with repercussions in everyday activities such as shopping, handling money and finding your way back. All these symptoms are accompanied by anxiety and loss of motivation. Symptoms worsen as the disease progresses *(Steinberg M. et al., 2008).* The finality of this disease consists in the inability of the person to take care of himself. Brain changes in Alzheimer's disease consist of the abnormal deposition of insoluble "plaques" of a fibrous protein known as amyloid and the presence of twisted fibers called "neurofibrillary tangles" in the brain *(Attems J., Jellinger AK., 2013).* The presence of abnormal plaques and tangles make it impossible for brain cells to function normally. Deficiency of the neurotransmitter acetylcholine also affects the learning and memory process *(Piggott MA., 2013).*

There is still much to be learned about the exact causes of Alzheimer's disease, but research has identified several potential triggers that may contribute to its onset. Among the most commonly studied triggers of Alzheimer's disease are: certain genetic mutations, such as the amyloid precursor protein (APP), presenilin-1 (PSEN1) and presenilin-2 (PSEN2) genes, and age. The risk of developing Alzheimer's disease increases as a person ages. In addition to the previously mentioned lifestyle factors, diet, smoking, lack of physical exercise can also play a role in the development of Alzheimer's disease. Poor cardiovascular health, including high blood pressure, high cholesterol, and diabetes, can also increase your risk of developing Alzheimer's disease. Traumatic brain injury accompanied by exposure to environmental toxins such as heavy metals or pesticides can increase the risk of this disease. Today it is known that an important and modifiable risk factor is education. Better education means better protection against dementia. A large number of dementia cases can be prevented by early intervention. Early changes in the blood vessel wall can be detected by early ultrasound methods or early biomarkers. These methods allow us to detect changes before the disease becomes clinically evident. Early detection of the disease allows timely management, and studies have shown that careful control of vascular risk factors can delay the onset or even reverse the progression of the disease *(Morovic S. et al., 2019).*

Although neuroimaging and biomarker panels facilitate the detection and severity of Alzheimer's disease, a definitive diagnosis can be made by post-mortem examination of the brain.

Among the hypotheses underlying the development of Alzheimer's disease is the cholinergic hypothesis, which claims that deficiencies in the brain's cholinergic system, involving the neurotransmitter acetylcholine, play a significant role in the development of Alzheimer's symptoms. The level of this neurotransmitter, acetylcholine, can be disturbed due to a deficiency of choline acetylase within the formations involved in the cognitive process. In 1970, a cholinergic deficit was discovered in the brains of patients diagnosed with Alzheimer's disease, mediated by deficits of the neurological enzyme, choline acetyltransferase. This, along with the recognition of the role of

acetylcholine in memory and learning, led to the cholinergic hypothesis of Alzheimer's disease *(Whitehouse PJ., 1998).*

Then comes the amyloid hypothesis which dates back to the 1990s. This postulates that the onset of Alzheimer's disease is due to abnormal processing of the large amyloid precursor protein (APP) to form β-amyloid (Aβ) *(Hardy J., Selkoe DJ., 2002)* . Aβ is a peptide formed from amyloid precursor protein (APP), as previously mentioned, by the enzymes β- and γ-secretase *(Murphy MP., LeVine H., 2010).* Aβ can then trigger a cascade that leads to synaptic impairment and neuronal loss and ultimately to the pathological manifestations of Alzheimer's disease, the hallmarks of which are amyloid plaques and neurofibrillary tangles (NFTs) composed of hyperphosphorylated τ protein, with the final result being neurodegeneration *(Serrano-Pozo A. et al., 2011).*

Another hypothesis in the occurrence of Alzheimer's disease is the tau protein hypothesis. Tau protein, from the composition of neurons, has the role of stabilizing microtubules in the cellular cytoskeleton and facilitating intracellular signaling processes *(Mandelkow E.M., 1998).* Its hyperphosphorylation causes tau protein to accumulate in these masses, in the form of neurofibrillary tangles, inside nerve cell bodies. The tangles interact incorrectly with cellular proteins, preventing them from performing their normal functions. Hyperphosphorylation occurs downstream of β-amyloid, with research suggesting that the accumulation of β-amyloid may initiate this process *(Bloom GS., 2014).* Hyperphosphorylated tau dissociates microtubules in neurons compromising axonal transport and diminishing synaptic function. In addition, there is evidence that toxic taul can increase β-amyloid production through a feedback loop mechanism *(Huang HCG., Jiang ZFF., 2009).*

Vascular disease, diabetes and hyperinsulinemia, apolipoprotein gene, neuroinflammation represent other important etiological mechanisms in the occurrence of Alzheimer's disease.

Given that the doctor must make a precise diagnosis, specific to Alzheimer's disease, clinicians call for a correct identification and quantification of biomarkers specific to this neurodegenerative disorder. Thus an ideal biomarker should meet criteria of high specificity and sensitivity, reproducibility, repeatability and minimal costs (Spiller R.C., 2011). A clinical diagnosis of Alzheimer's disease is inaccurate even among experienced investigators in about 10% to 15% of cases, and biomarkers could improve diagnostic accuracy. Plasma biomarkers for AD risk can be identified in cerebrospinal fluid, YKL - chitinase-like protein 1 3 or human cartilage glycoprotein 39) cholesterol, followed by homocysteine or those proteins associated with the inflammatory process, including C-reactive protein, IL-1β, TNF, IL-6 and transforming growth factor β (Hansson O. et al., 2007). Biochemical parameters from fecal matter also play an important role in the early diagnosis of AD, highlighting the importance of the brain-gut axis in the way of permanent bidirectional communication between the central nervous system (CNS) and the gastrointestinal tract. The body of evidence supporting the influence of the gut microbiota in brain-gut interactions across all periods of development has increased, starting from early life to neurodegeneration, as well as interactions that occur at different levels, starting from the intestinal lumen to the CNS *(Dinan TG., Cryan JF., 2017).*

Diabetes mellitus refers to a group of metabolic diseases with a common feature, namely, an increase in the level of glucose in the blood above the allowed limit, that is, a state of hyperglycemia *(Harreiter J., Roden M., 2019)* associated with a risk high occurrence of microvascular and macrovascular diseases *(Zaccardi F. et al., 2016).* This severe hyperglycemic state causes different symptoms such as polyuria, polydipsia, fatigue as well as

loss of performance, accompanied by unexplained weight loss, visual disturbances and susceptibility to infections in ketoacidosis, hyperosmolar syndrome with risk of coma *(Harreiter J., Roden M. , 2019).*

Recently, scientists discovered a new form of diabetes, type 3 diabetes, also defined as metabolic syndrome. It produces defects related to insulin resistance of the brain, defects in insulin signaling pathways, accompanied by the accumulation of neurotoxins, the presence of stress at the level of neurons, so that finally neurodegeneration occurs *(Nguyen T. et al. 2020; Caberlotto L. et al., 2019).* Diabetes affects memory processing, also brain morphology, i.e. brain atrophy, as well as synaptic transmission.

Tests to diagnose diabetes include:

- fasting serum glucose determination test;
- determination of glycosylated hemoglobin;
- glucose tolerance test;
- random plasma glucose testing *(American Diabetes Association, Standards of medical care in diabetes, 2007).*

The glycosylated hemoglobin test is the most suitable in screening and diagnosis due to the following characteristics:

- non-compulsory fasting 8 hours before harvesting;
- suggests the average blood sugar levels of the last three months, according to the lifespan of the red blood cells;
- it is a standardized and safe method;
- not affecting values by stress or associated diseases, diets or intense efforts *(Saudek CD. et al., 2008).*

A new marker, a new approach in diabetes screening, is glycosylated albumin. Since glycosylated hemoglobin does not accurately provide the accelerated changes in glycemic control, in patients whose hemoglobin varies or in those with different types of anemia, nor information with reference to postprandial plasma glucose, but only the average level of plasma glucose, the need to approach of a new marker in diabetes management. It is glycosylated albumin *(Koga M. et al., 2011; Miyazaki A. et al, 2012).* Among the characteristics that recommend it are the high proportion of albumin in the total number of plasma proteins (60%), as well as the duration of life (21 days). Thus, glycosylated albumin can indicate the average glycemic values of the last three weeks, unlike glycosylated hemoglobin, three months, allowing the specialist doctor to more quickly evaluate the effectiveness of the treatment prescribed to the patient in question.

Fructosamine continues the line of innovative markers used in diabetes screening. Fructosamine is a general term given to any glycated protein. In the glycation reaction, the aldimine is produced first, unstable form, then stable ketoamine is formed, and by binding the side chain of ketoamine, its structure resembles the structure of fructose, hence the name fructosamine. With the help of fructosamine, glycemic control can be managed at an interval of 2-3 weeks, not being influenced by anemic states or fluctuating hemoglobin. Basically, it is an intermediate between glycosylated hemoglobin and glycosylated albumin *(Armbruster DA., 1987).*

1,5 - Anhydroglucitol (1,5-AG) represents another marker, more recently, in the control of short-term blood sugar as well as in postprandial hyperglycemia, practically used in the monitoring of glycemic fluctuations

in patients with type 2 diabetes. Studies in the literature specialist demonstrates that this innovative marker can be successfully used only in well-monitored diabetic patients, but which cannot be correlated with oxidative stress *(Kim MJ. et al., 2013).* It is a natural monosaccharide from the diet. Its maintenance in the body is done by renal excretion and reabsorption. Its reabsorption from the proximal tubule is competitively inhibited by glycosuria as serum glucose exceeds the 180mg/dL threshold. So, it could be considered not a marker of glycosuria, with the role of identifying very well the rapid variations of postprandial blood glucose *(Chan CL. et al., 2017; Sato A., 2014).*

The question of whether Alzheimer's disease is type 3 diabetes is helped by Zina Kraner's 2009 study, in which Alzheimer's disease is described as a neurodegenerative disorder based on the idea that insulin can control how neurotransmitters are released in the synapses and it would also be the one that activates the signaling pathways responsible for learning and long-term memory *(Kroner Z., 2009).* Neurotoxins such as amyloid-beta-derived diffusible ligands (ADDL) can affect insulin signaling *(Viola KL. et al., 2008).*

Insulin resistance and high insulin levels in type 2 diabetes outside the central nervous system induce a decrease in insulin in the brain with subsequent impairment of signaling pathways associated with learning and memory. Decreased insulin in the brain affects neuronal survival, energy metabolism, and plasticity, thus affecting cognition *(Li L., Hotscher C., 2007).*

CHAPTER 2. MATERIALS AND METHODS OF RESEARCH

Animal models

The current study uses the species Carssius auratus (caras, goldfish or Prussian carp (eng.)), used with success in other studies to highlight spatial memory *(Thangaleela S. et al., 2021)* duet o the forebrain, telencephalon, formation involved in the cognitive process *(Yamamoto N., 2009)* and which can be assimilated to the limbic regions in mammals, which are responsible for both spatial memory and those related to different types of behavior *(Rodriguez F. et al., 2002).* It has also been used as a toxicological model *(Lu Q. et al., 2018)* to study oxidative stress and other metabolic mechanisms as a result of exposure to different concentrations of sucrose *(Falfushynska H. et al., 2019)* .

Human patients

The research in this study was focused on patients from the Institute of Psychiatry "Socola" Iași, with the diagnosis of dementia and diabetes in order to observe how dementia degrades, in correlation with diabetes. All steps were taken after all ethical aspects were approved with the collaborating institution beforehand. In national legislation, the obligation of computerized consent for research is fixed by law 46/2003 on patient rights. Article 19 of Law 46/2003 provides the following "The patient's consent is mandatory in the case of his participation in clinical medical education and scientific research. People who are not able to express their will cannot be used for scientific research, except for obtaining consent from the legal representative and if the research is also done in the interest of the patient"

The research methods used in animal studies focused on the following behavioral tests:

- the T-maze test to evaluate spontaneous exploratory behavior and short-term memory;
- light/dark preference test to assess anxiety;
- the mirror test for evaluating aggression;social preference assessment test.

In patient studies, with the aim of correlating cognitive decline with the severity of diabetes, the following parameters were discussed: form of dementia, MMSE score (minimum mental examination score), diagnosis of diabetes, values of biochemical parameters: blood sugar, cholesterol , triglycerides, as well as psychotropic medication, and for diabetes, if it has been prescribed.

Behavioral tests are used to assess cognitive or affective-emotional behavioral changes, as well as to obtain a greater degree of anxiety and depression in test animals.

The first behavioral test used in the present study is the T-maze test (fig.1) to evaluate spontaneous exploratory behavior and short-term memory in which the parameters used were:

1. The time spent in the three arms, specific to exploratory behavior, a parameter based on the animal's tendency to explore;
2. The number of entries in the three arms of the maze, also specific to exploratory behavior;
3. The sequence of left-right alternations (turns) performed during the test, relevant to short-term spatial memory.

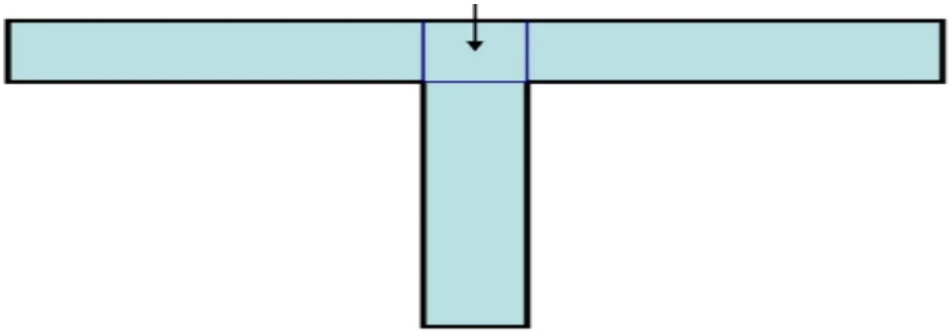

Fig. 1. T-maze used to assess spontaneous exploratory behavior and short-term memory (*d'Isa Raffaele et al., 2021)*

The light/dark preference test (fig.2) for assessing anxiety. The behavioral indicators analyzed were:

1. the entering latency into the illuminated compartment;
2. the time spent in the illuminated compartment (also calculated as a percentage of the total time allocated to the test) and the average duration of stay in it;
3. the number of passages between the two compartments;
4. risk assessment behavior, represented by the number of fast entries into the dark side and partial entries into the light compartment.

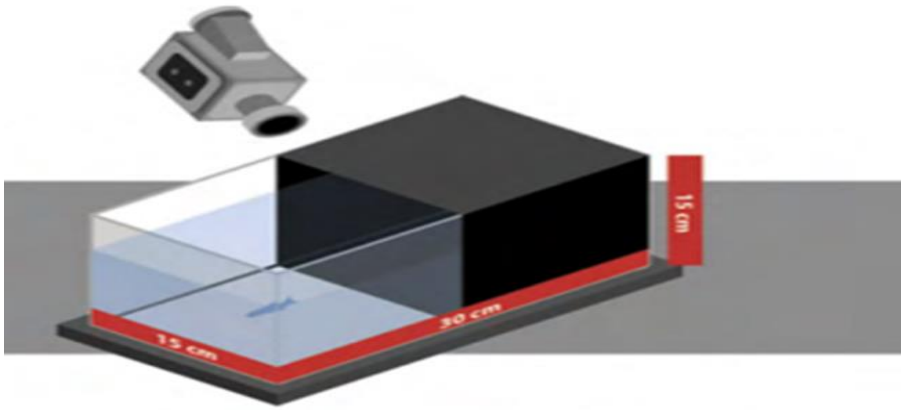

Fig. 2. Apparatus used to assess anxiety in the light/dark test *(Danielle L. Champagne, 2010)*

Aggression Mirror Test (fig.3). The behavioral parameters followed were:

1. mirror bite frequency: how many times the fish bites/comes into direct contact with the mirror;
2. duration of mirror biting: time spent biting/in direct contact with the mirror;
3. frequency of approaching the mirror: the number of crossings of the line denoting the area of approaching the mirror, but without contact with the mirror;
4. contact latency: the time until the first contact with the mirror;
5. other behavioral parameters reflecting the appearance of the state of anxiety such as the duration of freezing/immobility.

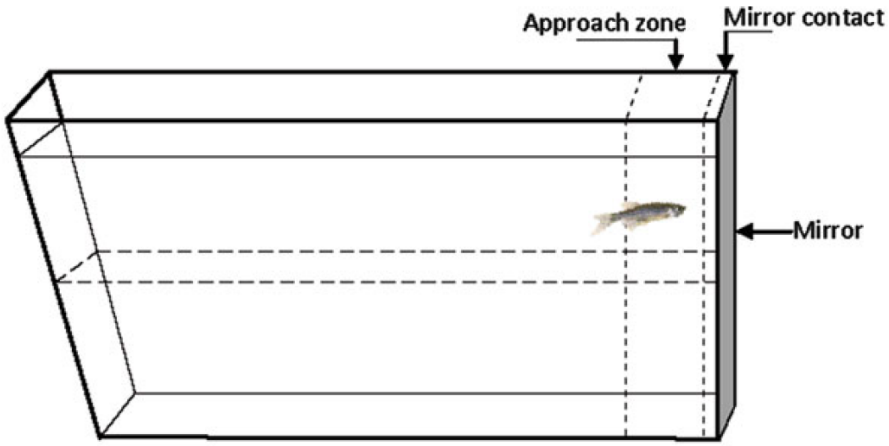

Fig. 3. Device used to evaluate aggression in the mirror test *(Pham M., 2012)*

The social preference test (fig. 4). The behavior of the fish was analyzed taking into account the following parameters:

1. the latency of entering into contact with the social target: the duration until the first contact of the fish with the social target;
2. the time of effective interaction with the target fish;
3. the number of entries and the time spent in the social interaction area, in the central area and in the remote area;

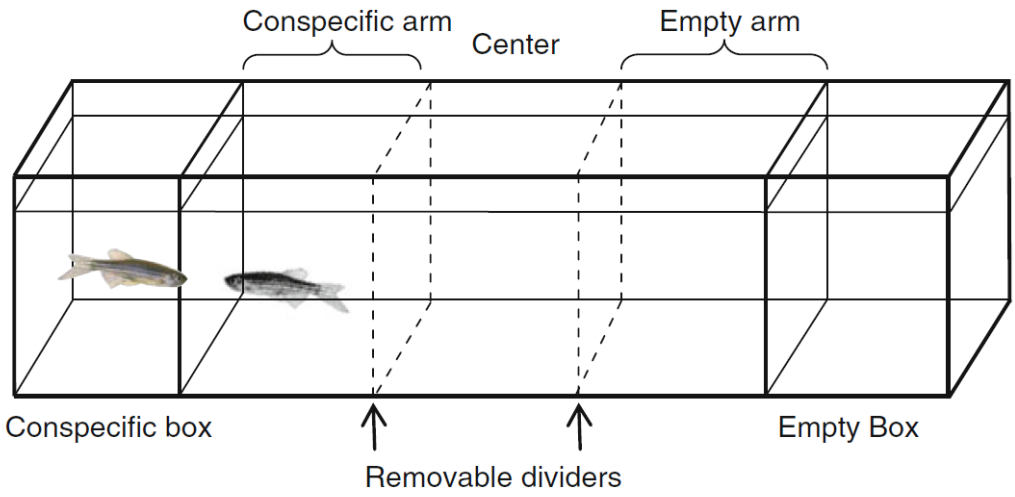

Fig. 4. Device used to assess social preference *(Pham M., 2012)*

The last parameter, from the T-maze test for evaluating spontaneous exploratory behavior and short-term memory, is based on a mathematical model based on combinations of four turns called tetragrams. 16 such possible combinations can be formed, but only two of them have the best statistical probability, SDSD (left-right-left-right), DSDS (right-left-right-left) and which reflects best the specific strategy for spatial orientation. The strategy is based on alternations, the way the fish turns, left then right or vice versa, a conscious choice based on memory, and not on repetitions that rely on reflex actions. Although fish process information differently from rodents and have a different orientation in space compared to them, they still have in common the desire to find an exit from the apparatus, to take refuge in a place where they feel safe. This model was stated as early as 2018-2019, with applicability from 2021, where Cleal and colleagues validated the model in fish, mice and humans. They also showed that in a normal individual, without his memory being affected, the combinations of the alternating type have an average value of 40%, the rest being of the repetitive type 60% *(Cleal M., Parker M.O. 2018).*

The research methods used in the studies on human patients from the Institute of Psychiatry "Socola" Iași, with the diagnosis of dementia took into account the following parameters: the form of dementia, the MMSE score, the diagnosis of diabetes, the blood glucose value, the cholesterol value, the triglycerides, psychotropic medication, medication for the type of diabetes discovered, with the aim of correlating the degree of cognitive degradation with the severity of diabetes as well as studying how the specific medication for Alzheimer's disease and diabetes influences the pathological picture of these diseases and the interactions between them.

CHAPTER 3. RESEARCH RESULTS

Results and discussion

Regarding the first parameter of spontaneous exploration, the time spent in each of the three arms of the maze, in the first experiment, a uniform trend was observed in the case of the control and sucrose groups, and in the case of the fipronil and sucrose with fipronil groups a significant preference was found to explore only one of

the arms (the left) at the expense of the other two (fig.5). The basis of this behavior could be the state of hyperexcitability induced by fipronil *(Gupta RC., Anadon A., 2018).*

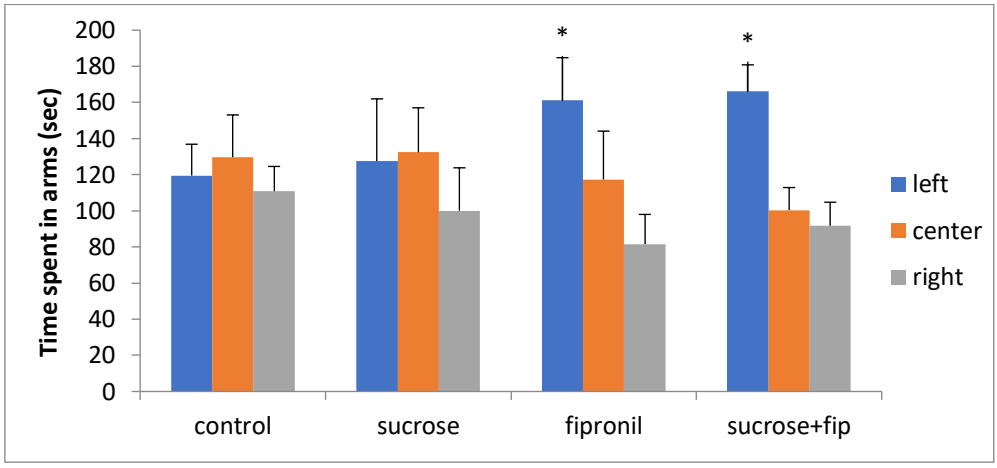

Fig. 5. Time spent in each of the three arms of the maze after acute exposure to sucrose, fipronil and sucrose+fipronil. Data were expressed as mean ± SD (n = 14, *p<0.05 vs. control, **p<0.01 vs. control, ***p<0.001 vs. control).

The exploratory and locomotor activity expressed as the total number of entries in the arms of the maze registered a decrease for all three treated groups vs control, a significant difference was observed in the case of the fipronil group, accompanied by a chaotic swimming which highlights the disruption of the CNS, of the activity movements under the action of fipronil (fig.6). There are studies in the specialized literature that claim that exposure to fipronil, in experiments performed on rats, affected nigrostriatal dopaminergic neurons with consequences on locomotion, motor coordination, memory and social interaction *(Sindhu KM. et al., 2006)* .

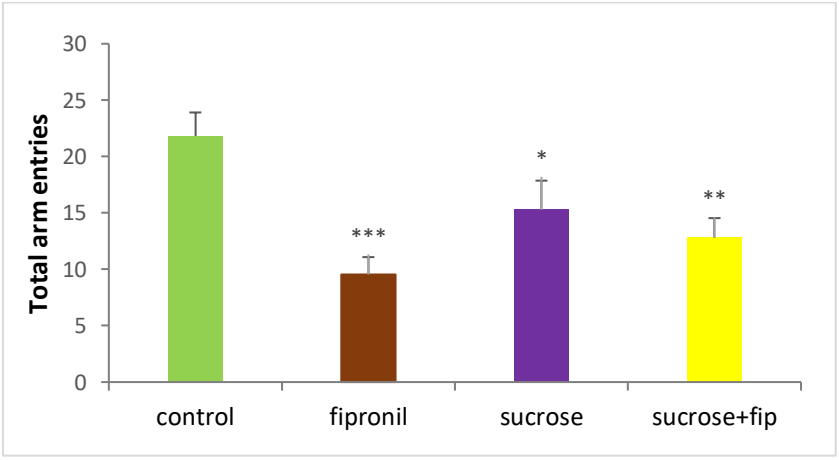

Fig. 6. Total number of maze arm entries after acute exposure to sucrose, fipronil, and sucrose+fipronil. Data were expressed as mean ± SD (n = 14, *p<0.05 vs. control, **p<0.01 vs. control, ***p<0.001 vs. control).

Learning is considered a first stage of memory because it involves the accumulation of new knowledge, and memory involves the highlighting, highlighting of this learned information. These processes are based on certain areas in the brain that contain numerous GABA receptors: the amygdala, the hippocampus and the entorhinal cortex. Preferably the hippocampus, which is a GABAergic system. By disrupting these receptors due to the toxic action of GABA antagonists cognition can be impaired *(Lynch MA., 2004).* So, the GABA receptor

subject to the mechanism of action of fipronil may be the basis of unusual changes or alterations in the processes that lead to learning, as well as those mechanisms that participate in the synergistic formation of cognitive consolidation and spatial memory *(Izquierdo I., Medina JH., 1991; Davis M., 1994, Godinho AF. et al., 2016).*

Referring to the previously mentioned literature data, we can appreciate the decrease in the sequence of arm alternations, a parameter considered to reflect spatial working memory, a form of short-term memory (fig. 7), for the fipronil group and with statistical significance for the s+f group, compared to the percentage of alternations of the control group, precisely due to the damage to areas in the brain with a large content of GABA receptors (amygdala, hippocampus, entorhinal cortex), involved in the cognitive and implicit process of spatial memory.

We mention that the percentage of alternations obtained in the case of the control group is 45%, close to the standard value in the specialized literature (40% for individuals with an unaffected memory).

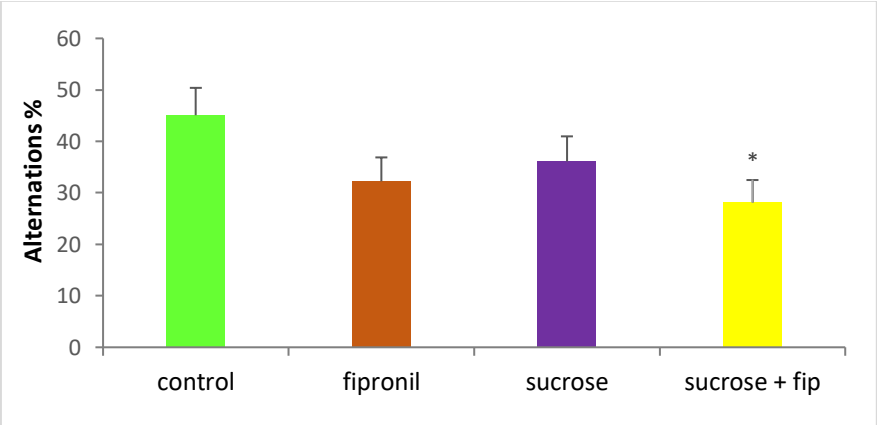

Fig. 7. Effects on short-term memory assessed by arm alternation sequence after acute exposure to sucrose, fipronil and sucrose+fipronil. Data were expressed as mean ± SD (n = 14, *p<0.05 vs. control, **p<0.01 vs. control, ***p<0.001 vs. control).

In the second experiment, during the T-test, for the number of arm entries after exposure to progressive doses of sucrose (fig. 8), no statistically significant changes were recorded between groups. Visual decreases between the 5.5 mM sucrose group and the control, correlated with episodes of sustained freezing (freezing state, total immobility) in the same group in the mirror test.

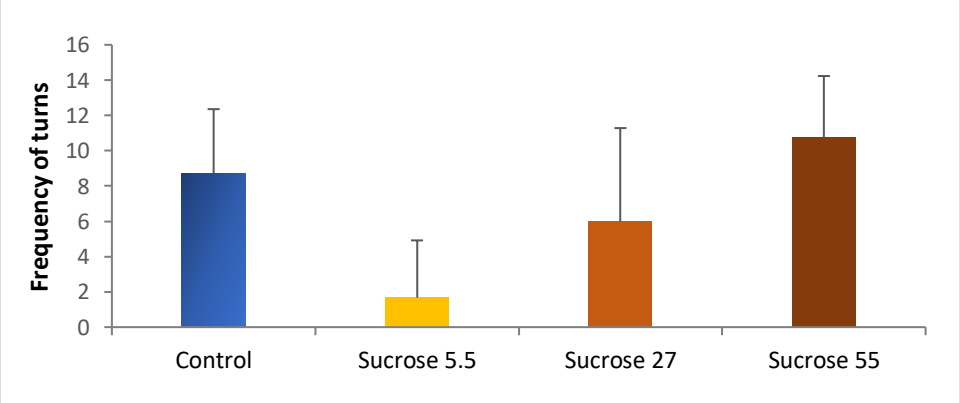

Fig. 8. Number of arm turns after exposure to progressive doses of sucrose; 5.5 mM; 27.75 mM; 55.5 mM. Data were expressed as mean ± SD (n = 9)

The percentage of arm alternations, the indicator reflecting short-term memory, registers a statistically significant decrease for the maximum concentration sucrose group vs. control (fig.9), no significant differences being observed between the other two groups of sucrose, highlighting the alteration of cognitive performance by exceeding a certain threshold of carbohydrate metabolism. This trend could be explained by the *Kroner Z., 2009* study in which the similarity of Alzheimer's disease with diabetes was supported, which could be considered a type 3 diabetes.

The term type 3 diabetes was coined in 2005 by Suzanne de la Monte, a neuropathologist and associate professor of Pathology and Medicine at Brown Medical School. This, following post-mortem examination of the brains of AD patients, noted that the disease could be considered a neuroendocrine disease associated with insulin signaling. Alzheimer's disease has been called type 3 diabetes because it includes elements of type 1 and 2 diabetes, with both decreased insulin production and resistance to insulin receptors *(Rivera EJ. et al., 2005; from Monte SM et al., 2006)*. Data from the literature show an 80% decrease in the number of insulin receptors in AD patients compared to normal subjects. It was also discovered that the ability of insulin to bind to receptors was compromised. So patients with AD have less insulin and fewer insulin receptors than patients without AD, and correcting insulin levels improves cognition. Insulin binds to insulin receptors in the brain, most of which are located in the cerebral cortex, olfactory bulb, hippocampus, cerebellum, and hypothalamus. The existence of insulin receptors in cognitively relevant areas of the brain leads us to consider the association between insulin and cognition *(Craft S., Watson GS. 2004).*

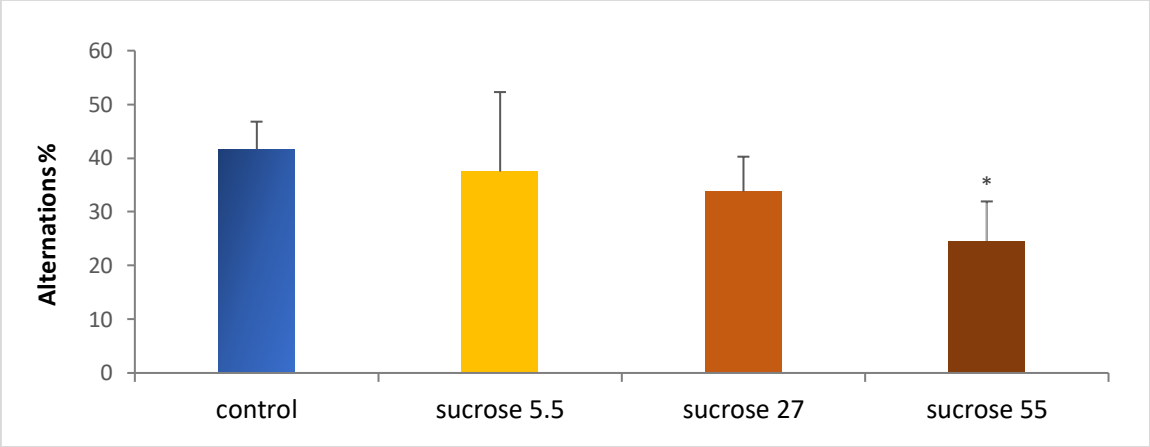

Fig. 9. Effects on short-term memory assessed by arm alternation sequence after exposure to progressive doses of sucrose 5.5 mM, 27.75 mM, 55.5 mM. Data were expressed as mean ± SD (n = 9, *p<0.05 vs. control).

The other behavioral tests presented in the first experiment highlight anxious-type manifestations for the fipronil and s+f groups in the light/dark test (fig.10). This conclusion is consistent with those published in the specialized literature. Thus, the article written *by Akhlaq Huussain, Gilbert Andira* and collaborators, argues what was previously claimed, in that zebrafish exposed to pesticides performed more rotation movements in the dark side than in the light side compared to the other groups, suggesting the possible anxiogenic effect of fipronil *(Hussain A., Audira G. et al., 2020).*

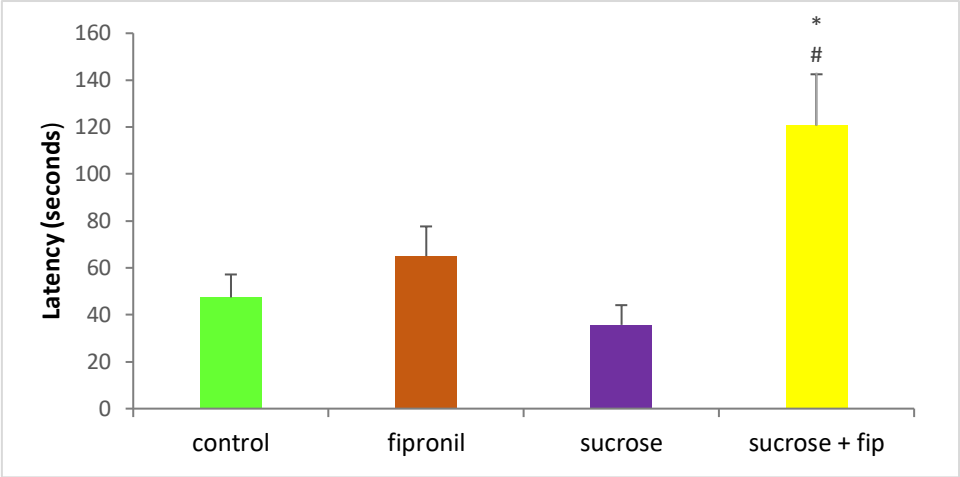

Fig. 10. Latency to enter the lighted compartment in the Light/Dark Preference Test after acute exposure to sucrose, fipronil, and sucrose+fipronil (n=14, *p<0.05 vs control #p<0.05 vs sucrose)

Anxious-type manifestations are observed in the same mixed group and in the mirror test, as well as aggressive-type manifestations for the other two groups (fipronil and sucrose) (fig.11). Regarding the mirror bite test, to assess the animal's boldness or, on the opposite end, its anxiety, the specialized literature provides data in this regard. The study of Audira G. and his collaborators provides data on the evaluation of the aggressive behavior of zebrafish under the action of different polluting, toxic substances following the boldness of the animal *(Audira G. et al., 2020).* Other studies provide conflicting data regarding aggression in animal models. Thus, in the study in which the pesticide used was ethanol, an inhibition of aggression was observed, due to the sedative effect of ethanol *(Audira G. et al., 2018).* Unlike another study present in the specialized literature, where other types of pesticides were used, and in which an opposite reaction to the previously mentioned one was observed, an increase in aggressive behavior, by modifying stress hormones as well as the genes involved in the regulation of aggressive behavior *(Liu ZH. et al., 2020).*

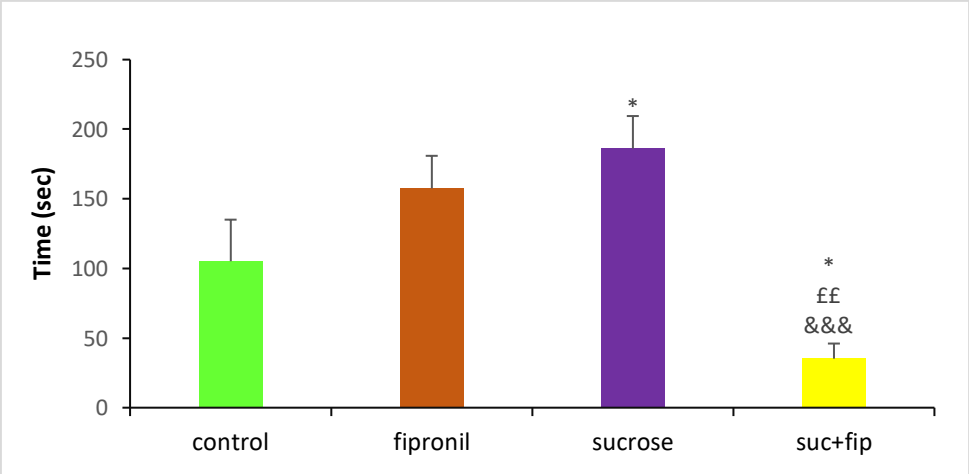

Fig. 11. Time spent in the mirror contact zone after acute single-dose exposure to sucrose, fipronil and sucrose+fipronil (n=14, *p<0.05 vs control, ff p<0.01 vs fipronil, &&&p < 0.001 vs sucrose)

The literature shows that exposure to fipronil affects dopaminergic neurons with consequences on locomotion, motor skills, memory and social interaction *(Sindhu K.M, 2006).*

For the second experiment, the anxiogenic effect of chronic sucrose is visible in the light/dark test for the 27.75 mM and 55.5 mM groups and the aggression set among the minimum concentration of 5.5 mM (fig.12). Researchers theorize that physical exercise reduces anxiety-type manifestations by mediating the hypothalamic-pituitary-adrenal axis (Fulk LJ. et al., 2004) and could in this case increase manifestations located at the opposite pole, of aggression, which could be a plausible explanation for the behavior shown by the group treated with 5.5mM.

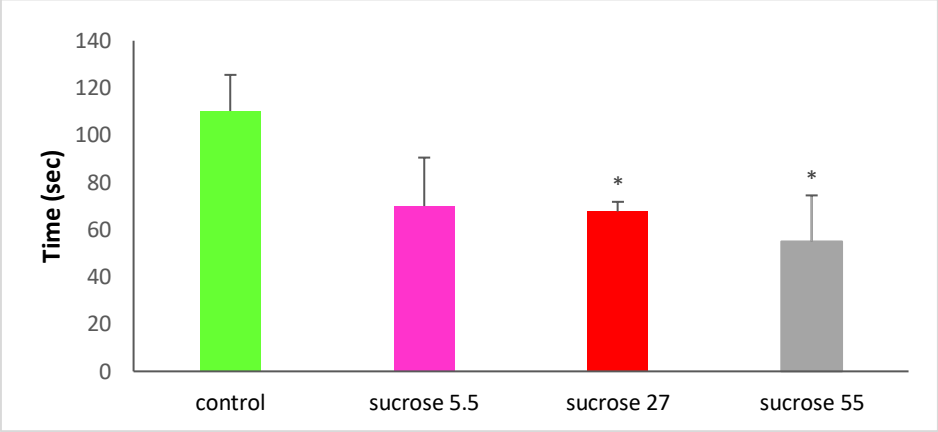

Fig. 12. Time spent in the illuminated compartment after exposure to progressive doses of sucrose; 5.5 mM; 27.75 mM; 55.5 mM (n = 9, *p<0.05 vs control)

Fishes also tend to display an approach-avoidance strategy. Although they show a desire to explore new environments, this is accompanied by states of anxiety, fear of new, potentially dangerous places. Thus, the fish will choose to stay in a comforting environment for them, i.e. the dark area, with short forays into the hostile environment, the light area *(Montgomery KC., 1955), (Toth, Miklos and Bojana Zupan, 2007).* In the light/dark test, hyperglycemic fish recorded a reduced number of crossings, also the time spent in the light zone was decreased accompanied by a lack of risk assessment manifestations (fig.13). All these phenotypes suggest anxiety-like behaviors attributed to high glucose levels in the body *(Capiotti KM. et al., 2014).*

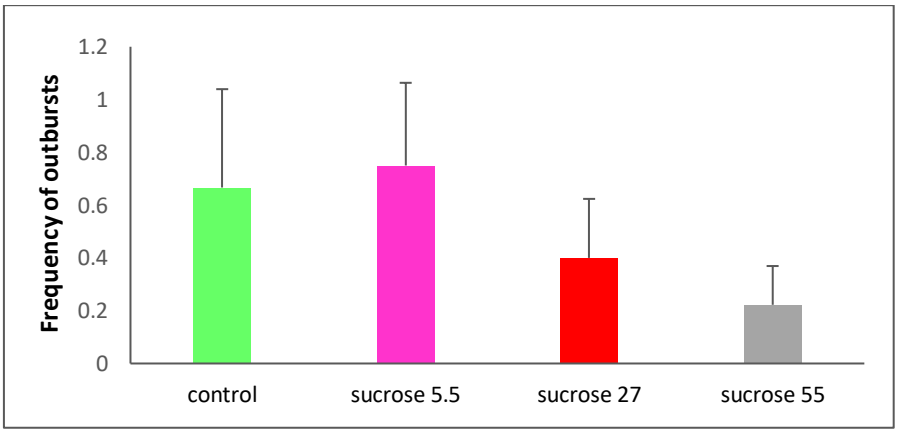

Fig. 13. Risk assessment behavior, represented by the number of rapid entries into the dark side and partial entries into the lighted compartment, after exposure to progressive doses of sucrose, 5.5 mM; 27.75 mM; 55.5 mM (n = 9)

In the mirror test, anxiety state installation observed for the 5.5 mM group is relevant vs control and vs and the second group 27.75 mM (fig.14). It is difficult to interpret the significance of these values, whether they reflect the reduction of motor activity or aggression, or the effects of internal regulatory mechanisms against metabolic imbalances. The possible explanation would be the installation of compensatory mechanisms against metabolic imbalances with implications on behavioral manifestations. This is also highlighted in the specialized literature, through the article written by Unai Galicia-Garcia and his collaborators, which explains the metabolic imbalances as the result of the disruption of the mechanisms involved in the process of insulin synthesis and release, as well as in its detection. Affecting glucose homeostasis through any of the previously mentioned mechanisms creates metabolic imbalances *(Galicia-Garcia U. et al., 2020).*

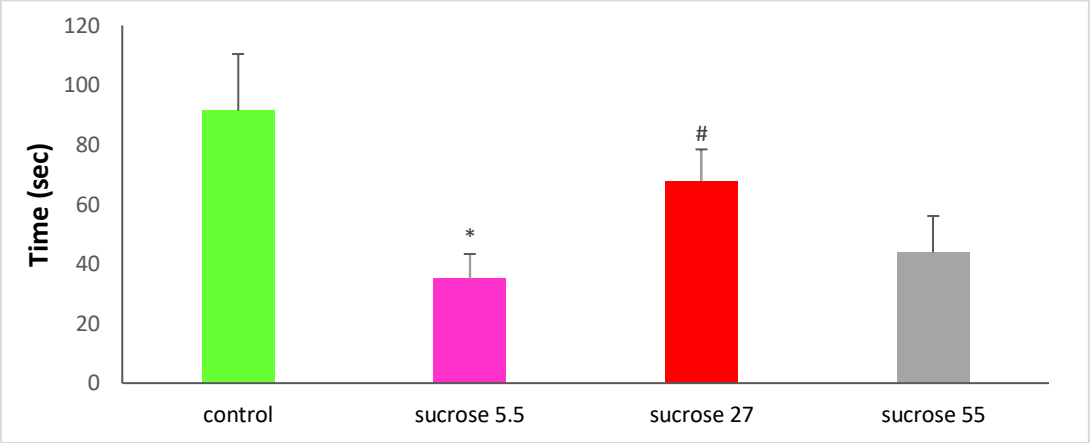

Fig. 14. The time spent in the area away from the mirror, after exposure to progressive doses of sucrose; 5.5 mM; 27.75 mM; 55.5 mM (n = 9, *p<0.05 vs control, #p<0.05 vs sucrose 5.5 mM)

Regarding the social preference test, the reduction of the social component is observed for the 55 mM sucrose group, and for the 5.5 mM group, rather an exploratory reduction than the social component. (fig. 15). Altered, decreased social preferences are difficult to interpret because they can also be attributed to a locomotor disturbance unrelated to anxiety. Genetic dysfunctions in muscles or motor pathways can have negative effects in motor neurons and even damage muscle cells, with repercussions on locomotion. So, it should be identified whether the altered social behavior would be as a result of a locomotor impairment or the result of an independent phenotype *(Norton WHJ. et al., 2019).* Social preference can be defined as the predilection of individuals to live near conspecifics *(Liu X. et al., 2016).* The data provided by the specialized literature suggest that the assessment of social preference can be influenced by both endogenous and exogenous factors. In turn, the endogenous factors can be divided into two categories, one in which reference is made to the individual characteristics of the tested fish, and the other category refers to the group characteristics of the individuals. Individual characteristics include age, gender and personality. The size, phenotype and degree of kinship between them are part of the group. Let's not forget the exogenous factors that can intervene in the affected social component, namely environmental conditions, light, volume of water in the device, brightness, temperature *(Ogi A. et al., 2021).* Many studies claim that zebrafish prefer to socialize with conspecifics that have similar phenotypes, age, and size. Not having exact knowledge of which of these factors could have influenced the social preference, we can only attribute it to the high glucose concentrations in the blood of the fish, knowing that diabetes can affect brain processes by inducing

depression-like disturbances in the central nervous system , anxiety and Alzheimer's disease *(Lakstygal AM. et al., 2019).*

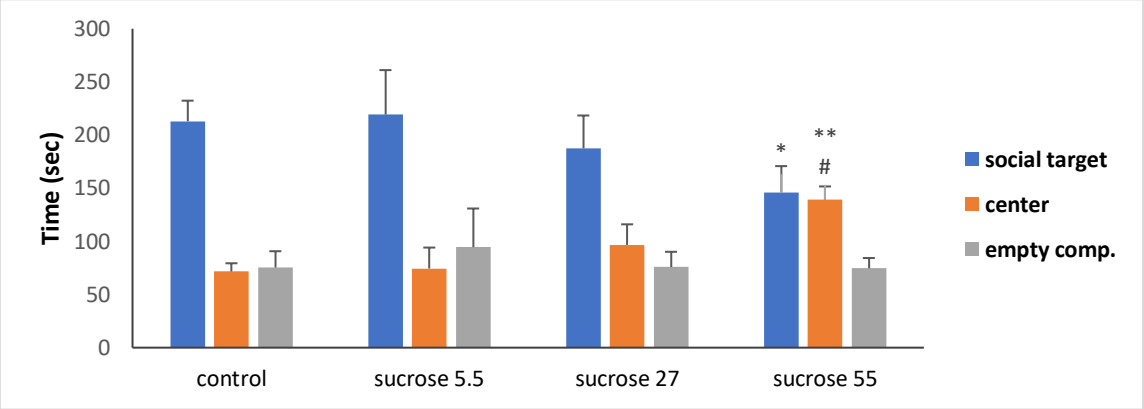

Fig. 15. Exploration time, in the Social Preference Assessment Test, after exposure to progressive doses of sucrose; 5.5 mM; 27.75 mM; 55.5 mM; (n = 9, *p<0.05 vs control; , **p<0.01 vs control; #p<0.05 vs sucrose 5.5 mM)

The second part of the study is a statistical evaluation of patients with diabetes and dementia states from Socola Hospital, from January to July 2022. Following the selection, a sample of 33 patients was assessed, who were divided into several categories according to the type of dementia (fig.16), in which the highest proportion is held by mixed dementia, medium form, followed by mixed dementia (Alzheimer's disease combined with vascular) and vascular dementia.

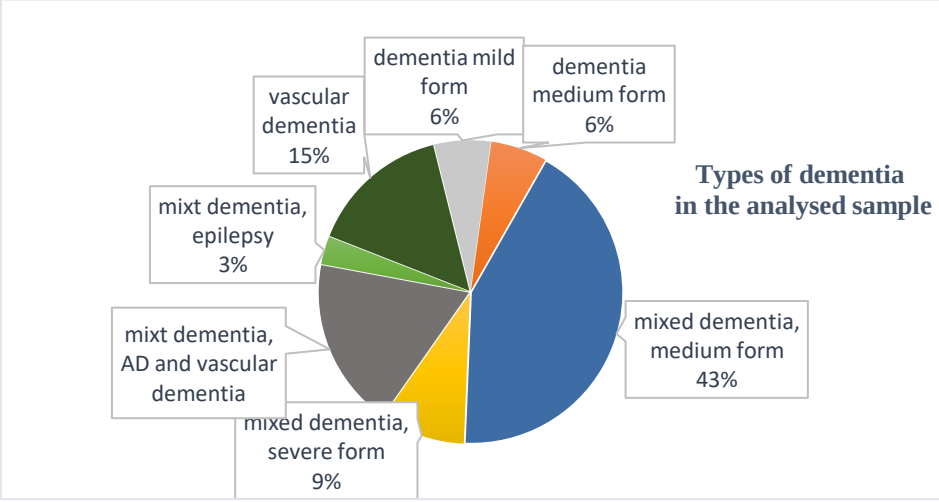

Fig. 16. Distribution of dementia types in the total sample of patients

The associated pathologies that are the subject of the present study are diabetes and obesity. Analyzing the prevalence of those with these pathologies from the total number of patients, we found the following data:

- ✓ in the group of moderate mixed dementia, 7% of patients had diabetes;
- ✓ in the group of mixed dementia, Alzheimer's disease and vascular dementia 16.6%;
- ✓ and in the vascular dementia group the percentage is 60% (fig.16).

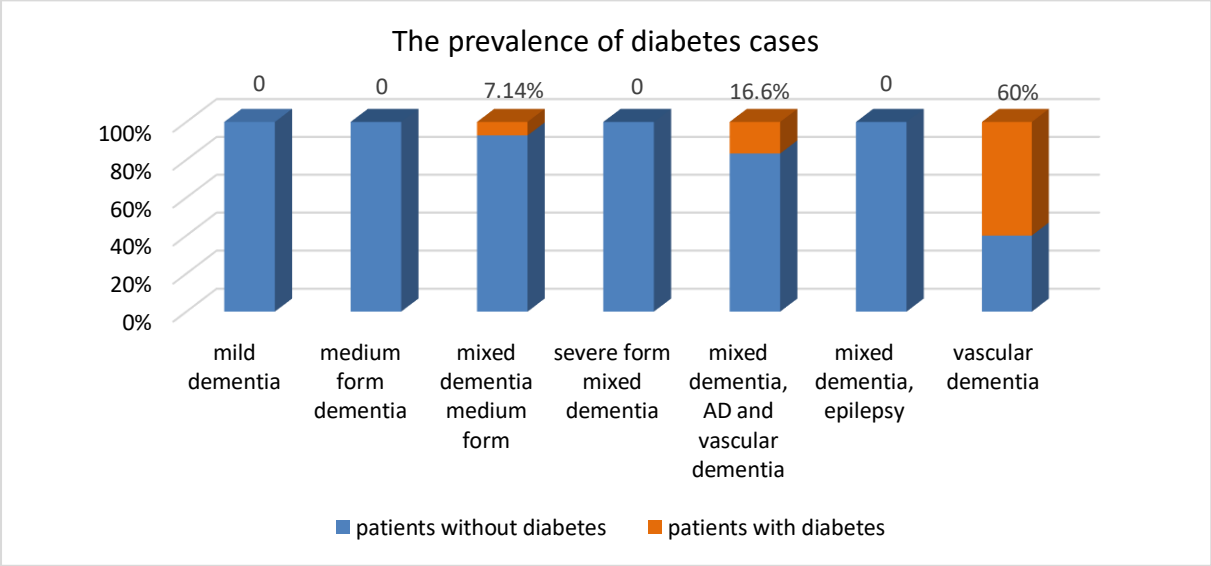

Fig. 17. Prevalence of diabetes cases

In the case of obesity, a high percentage, of 50%, is recorded in mild dementia types and 14.2% in mixed dementia, medium form (fig.18).

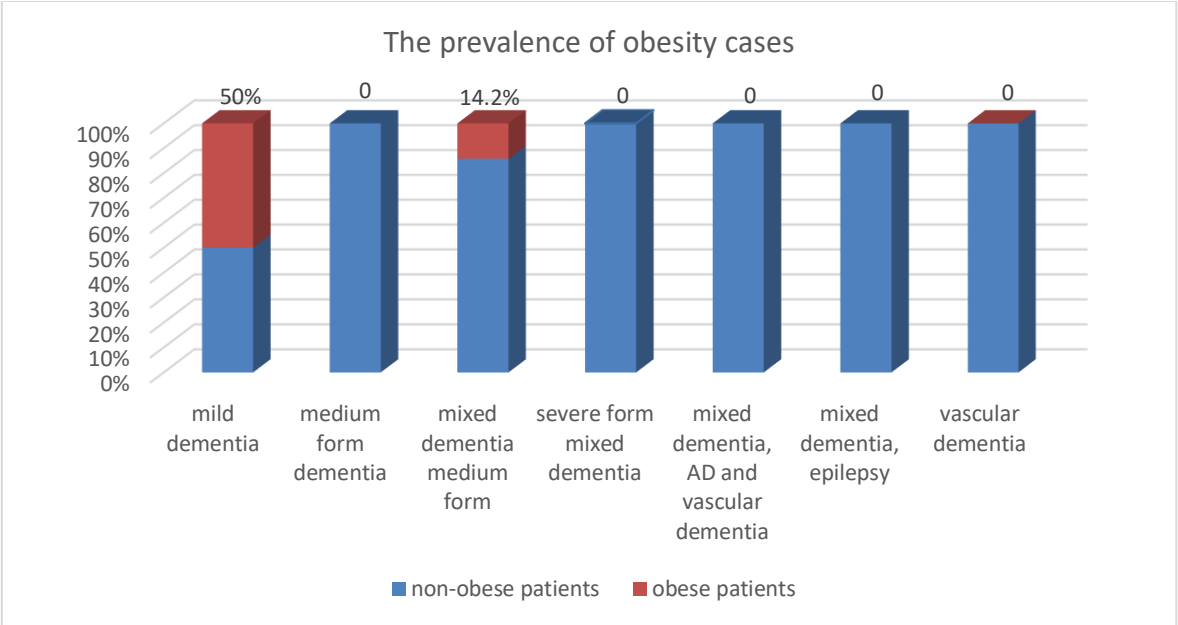

Fig. 18. Prevalence of cases of obesity and dyslipidemia

When highlighting the degree of severity of dementia according to the MMSE score, the mild dementia and vascular dementia are noted. The MMSE score is a well-known method questionnaire-based to assess the degree of cognitive decline (fig.19).

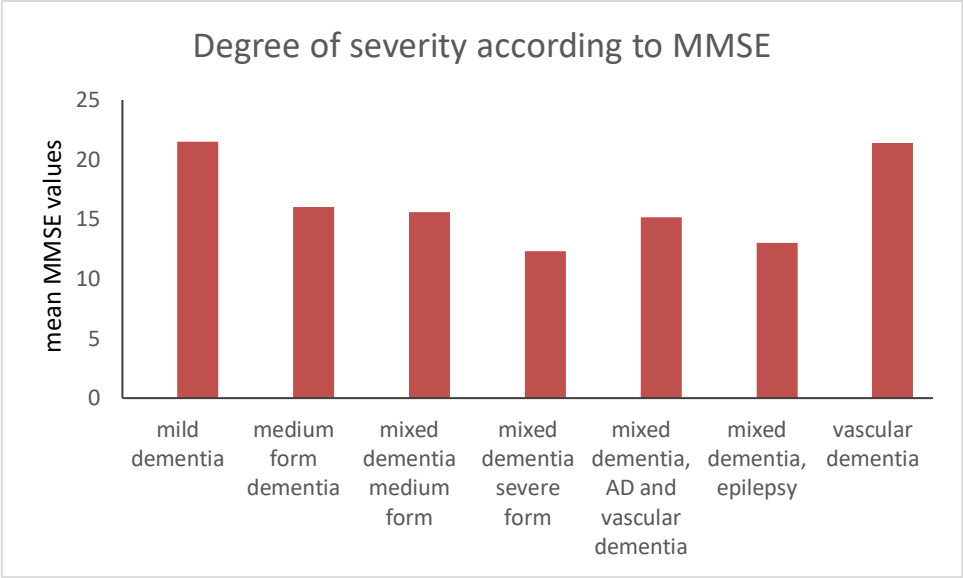

Fig. 19. Severity of dementia according to MMSE score

Blood glucose, cholesterol and triglycerides were analyzed for most of the patients in this study. Based on these parameters, we made a series of individual correlations, in which insignificant Pearson correlations were obtained for all mentioned parameters. The lack of correlations (fig. 20, 21, 22) could be attributed to the MMSE scores for dementia, there are categories of dementias with very high scores that do not exclude a major degree of damage, vascular dementias characterized by the increased incidence of diabetes cases, presenting, surprisingly, notes high on the MMSE scale close to normal values, without symptoms.

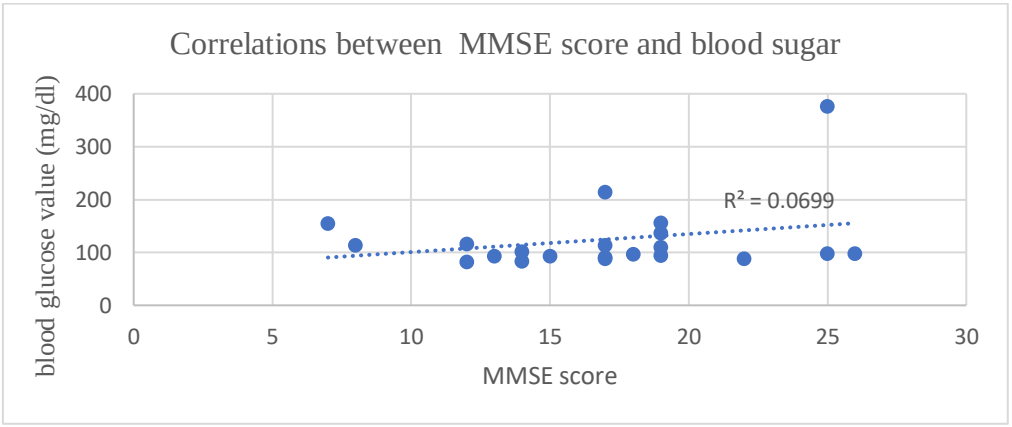

Fig. 20. Correlations between MMSE scores and patients' blood glucose levels

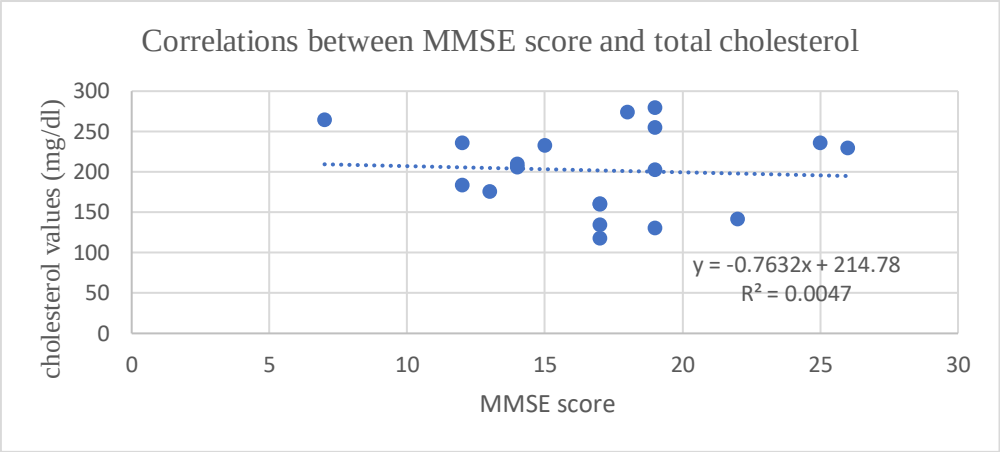

Fig. 21. Correlations between MMSE scores and patients' cholesterol

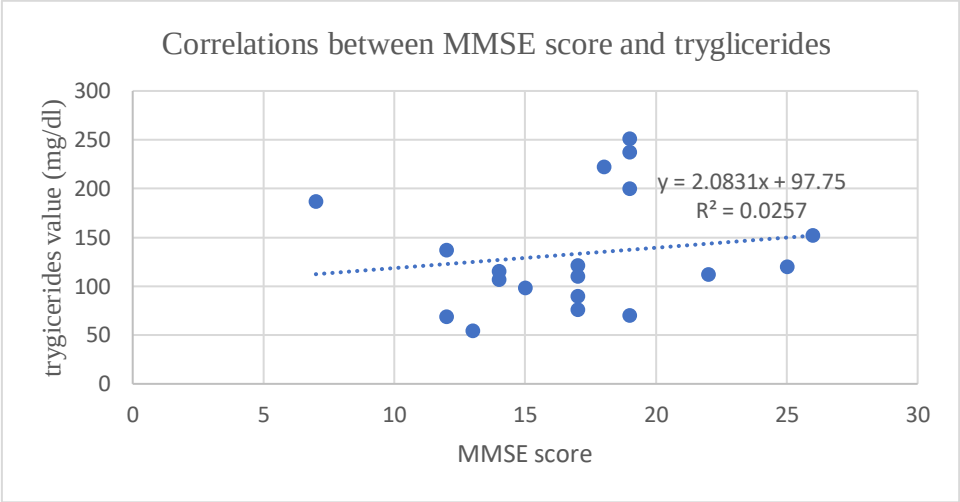

Fig. 22. Correlations between patients' MMSE scores and triglycerides

In this context, we applied another method of statistical analysis. We excluded patients without blood glucose due to missing data and made correlations between the percentage means of patients with diabetes by dementia type and the means of blood glucose, cholesterol and triglycerides, where we obtained significant Pearson correlations above 0.7 (fig. 23, 24, 25). Thus, according to the obtained correlations, there is only a certain type/subtype of dementia, namely vascular and mixed dementia (Alzheimer's and vascular disease), which is more vulnerable to cholesterol, diabetes or seems to be more vulnerable. These metabolic pathologies seem to be closely related to psychiatric manifestations.

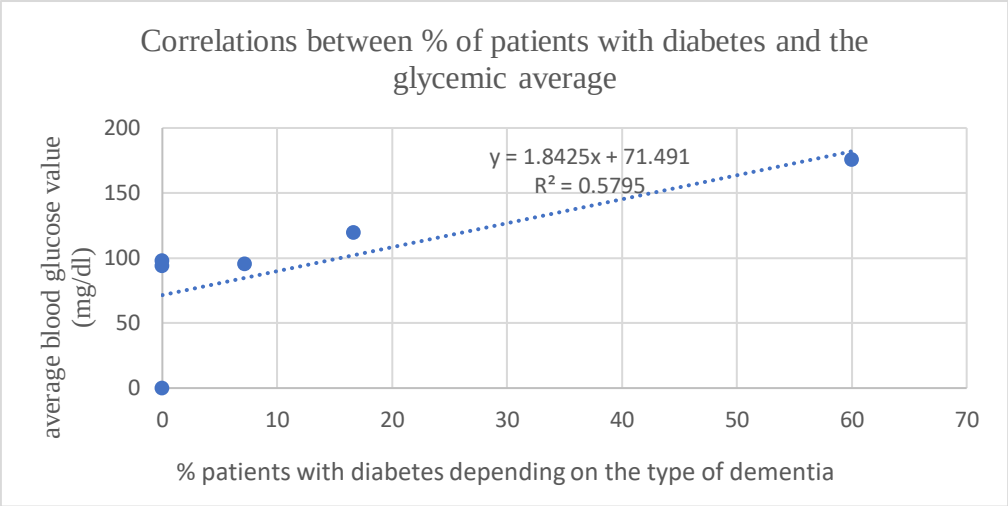

Fig. 23. Correlations between the percentage averages of patients with diabetes according to the type of dementia and the average blood glucose levels

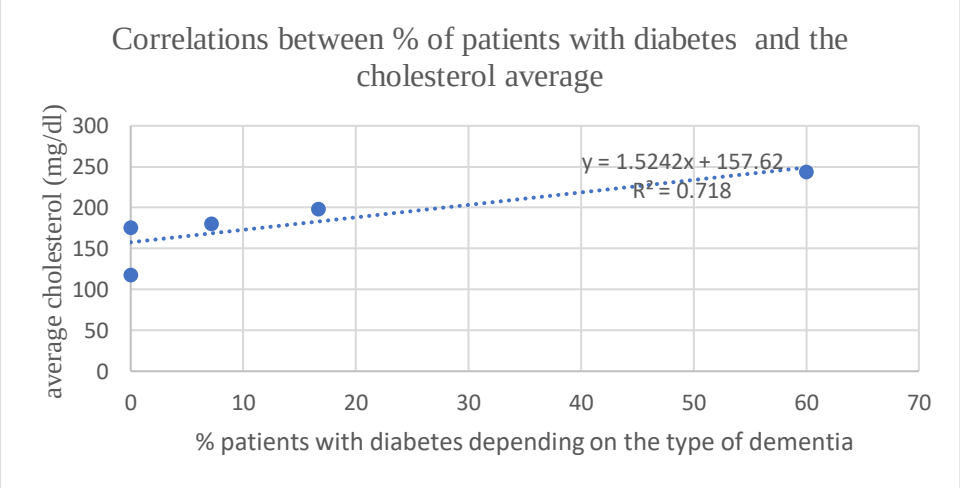

Fig. 24. Correlations between percentage means of patients with diabetes mellitus according to type of dementia and cholesterol means

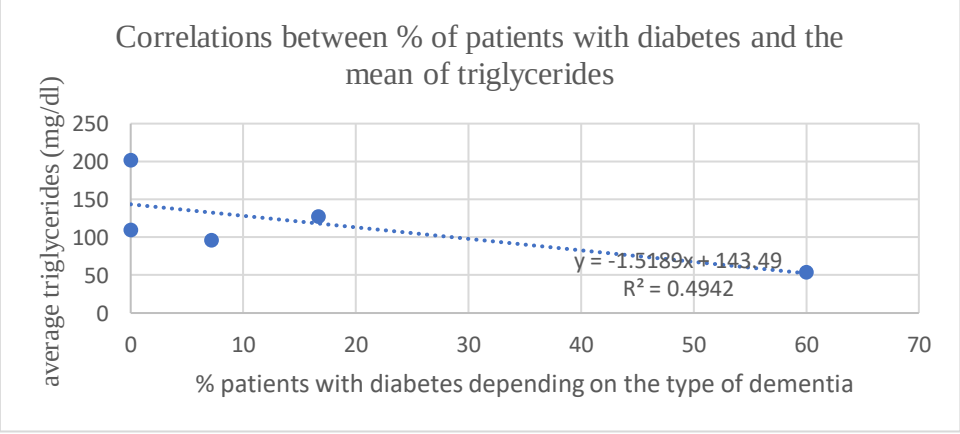

Fig. 25. Correlations between the percentage means of patients with diabetes mellitus according to the type of dementia and the means of triglycerides

It is also possible to observe the percentages in which patients follow psychotropic medications for the control of diabetes. The indecency of high blood glucose, above normal, is high among patients, 11 out of 29 show abnormal glycemic fluctuations (fig.26).

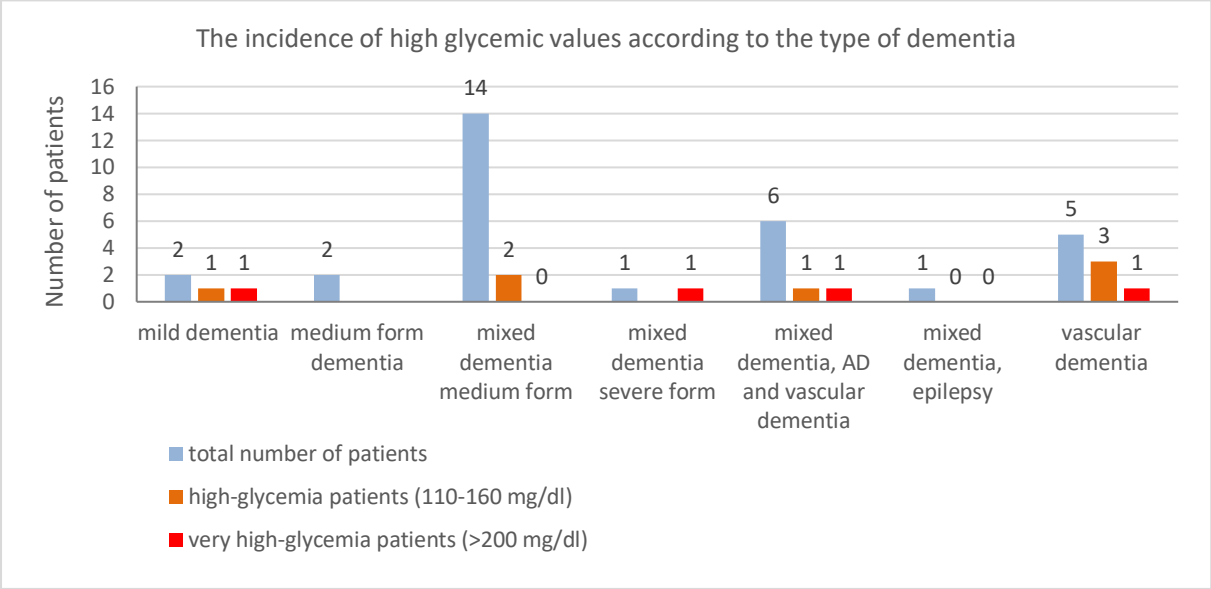

Fig. 26. Incidence of high glycemic values by type of dementia

Regarding the correlations between psychotropic medication and that for diabetes, these are more difficult to make for the data provided, as larger samples are needed as well as their collection at different intervals to monitor blood glucose dynamics in relation to psychiatric status.

It can be seen, however, that MMSE scores are generally better when patients are on psychotropic drug treatment. An exception occurs in the case of vascular type dementia where the MMSE score is very high (even close to normal values). This is also the type of dementia with the highest incidence of diabetes (fig.27).

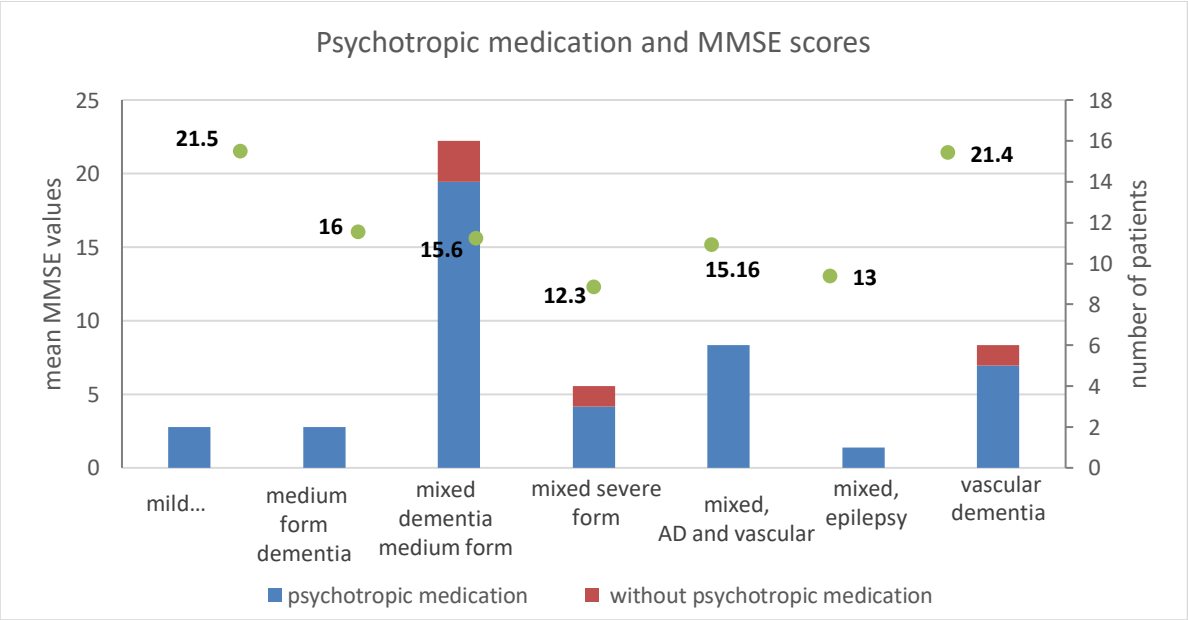

Fig. 27. Psychotropic medication and MMSE scores

Conclusions

- the animal models are characterised by the alteration of cognitive performance if exceeding a certain threshold of carbohydrate metabolism;

- the occurrence of short-term disfunctions, in the case of the sucrose+fipronil group, accentuates the pregnant effects induced by fipronil, against the background of a previous metabolic impairment.

- sucrose induces cognitive and affective behavioral alterations, marked by memory impairment, high anxiety and increased aggression.

- the cognitive impairment is also characterised by significant decreases in the percentage of alternating tetragrams in the case of the group treated with the maximum concentration of sucrose, emphasizing the idea of a metabolic influence.

- in small doses, sucrose is an energy source that induces a state of hyperactivity and even hyperexcitability, accompanied by unpredictable effects/freezing episodes or exacerbated anxiety.

- in the fish treated with sucrose in different concentrations the states of dominance and aggression may involve installation of compensatory mechanisms against metabolic imbalances with implications on behavioral manifestations.

- our data from the two experiments in the present study are in line with the current theories in the literature that the metabolic syndrome and diabetes could be the initial causes of Alzheimer's disease.

- our second study on human patients highlights correlations, mostly significant, between certain subtypes of dementia and associated pathologies (diabetes and obesity), namely those of the vascular type and mixed form (Alzheimer's and vascular disease) .

- the link between the two conditions, evident for only certain types of dementia, appears even if the analyzed sample is a small one, strengthening the hypothesis of the study.

- our results correlate with current theories regarding Alzheimer's disease as a particular form of diabetes, the so-called type 3 diabetes.

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