Ademi, M. (2022). The values and the differences of the serotonin levels in serum and brain tissue in an induced diabetic nephropathy white laboratory rat under the impact of perindopril and candesartan *MEDIS - Medical Science and Research, 1*(2), 23-30. doi: 10.35120/medisij010223a UDK: 577.175.8:[616.379-008.64-06:616.61

THE VALUES AND THE DIFFERENCES OF THE SEROTONIN LEVELS IN SERUM AND BRAIN TISSUE IN AN INDUCED DIABETIC NEPHROPATHY WHITE LABORATORY RAT UNDER THE IMPACT OF PERINDOPRIL AND CANDESARTAN

Majlinda Ademi[1*]

[1]Faculty of Medical Scienses, Study Program of General Medicine, University of Tetovo, Republic of N. Macedonia,
e-mail: majlinda.ademi@unite.edu.mk

Abstract: Angiotensin II is the primary vasoactive hormone of the renin-angiotensin-aldosterone system (RAAS) and plays a role in the pathophysiology of hypertension, heart failure, renal failure and other cardiovascular disorders. RAAS is activated in diabetic nephropathy (DN) and leads to more renal damage. Angiotensin converting enzyme inhibitors (ACEis) and angiotensin receptor blockers suppress this (ARBs). A large body of evidence demonstrates that, in addition to its typical activity as a hormone, Ang II is a neuropeptide produced by the central nervous system (CNS) that acts as a regulator of neurotransmission and nerve cell excitability. Peripheral serotonin is an endocrine component that promotes energy storage efficiency. Serotonin also enters the bloodstream and interacts with multiple organs, priming the body for energy storage by promoting insulin secretion and de novo lipogenesis in the liver and white adipose tissue. However, the actions of serotonin extend beyond neuronal communication in the CNS and enteric nervous system (ENS) to peripheral tissues. Serotonin mediates numerous nonneuronal processes such as bladder function, respiratory drive, hemostasis, vascular tone, immune function, and intestinal inflammation . The goal of this study is to see how the ACE inhibitor perindopril and the ARB AT1 candesartan, taken singly and in combination (double blockade), affect serotonin levels in the serum and brain tissue of Wistar rats with DN caused by streptozotocin (STZ). The levels of serotonin in the serum and brain of four experimental groups of animals were measured using an enzyme-linked immunosorbent assay (ELISA): a control group with DN, a group with DN treated with perindopril, a group with DN treated with candesartan, and a DN group treated with a combination of perindopril and candesartan. Perindopril (6 mg/Kg/day), candesartan (5 mg/Kg/day), and dual therapy with perindopril (3 mg/Kg/day) and candesartan (2,5 mg/Kg/day) were given orally every day for eight weeks, beginning four weeks after STZ was given, whereas the control group received just water. The rats were slaughtered at the end of the therapy so that the serum and brain tissue could be used to test serotonin levels. The results showed that blocking the renin-angiotensin system (RAS) with perindopril, candesartan, or their combination considerably decreased serotonin levels in the serum but dramatically elevated serotonin levels in the brain tissue in all groups.

Keywords: serotonin, serum, brain tissue, perindopril, candesartan.

INTRODUCTION

Serotonin (5-hydroxytryptamine; 5-HT) is a biogenic amine that has a variety of physiological functions, including basic mechanisms such as homeostasis, nutrition, immunity, glucose regulation, cardiovascular function, behavior, intestinal motility, and reproduction. About 90% of the total serotonin in the human body is found in the enterochromaffin cells of the intestine, where it participates in intestinal peristaltics. The rest is synthesized in serotonergic neurons in the central nervous system (CNS). It is a derivative of tryptophan (Hernandez, L.L.,2018). Tryptophan has 2 isoforms: TPH1 is responsible for the production of 5-hydroxytryptamin (5-HT) in peripheral tissues and TPH2 leads to the synthesis of 5-HT in the central nervous system. Platelets are probably the biological repository of circulating 5-HT. Plasma 5-HT levels are elevated in a variety of conditions, including hypertension and thrombosis (Fraer, M., & Kilic, F., 2015). 5-HT, a monoamine neurotransmitter, first recognised in 1948, has a wide range of functions in the CNS, including modulation of attention, cognition, behaviour, memory, and thermoregulation, as well as in the peripheral nervous system (PNS), where it regulates, for example, gastrointestinal (GI) motility, uterine contraction, vasoconstriction, and bronchoconstriction (Scotton, W. J., et al.,2019). It is usually called the happy hormone because it contributes to wellbeing and happiness. A decrease in its level has been seen in various mental illnesses like depression and anxiety (Bruta, K., et al., 2021). Chronic

*Corresponding author: majlinda.ademi@unite.edu.mk

renal failure (CRF) is a syndrome that develops as a result of a gradual, cumulative, and irreversible decrease in glomerular filtration rate (GFR) leading to uraemia. Diabetes mellitus (DM) is one of the most prominent risk factors for the development of HBI; diabetic patients account for 30 percent to 50 percent of all patients with "end-stage renal disease" (ESRD) globally (Ruiz-Ortega, M., et al., 2020). Diabetic nephropathy (DN) is becoming a leading cause of CRF and the need for hemodialysis in developed countries. The natural history of CFR or DN is weakened by the appearance of agents that block the renin-angiotensin-aldosterone system (RAAS). Renin-angiotensin-system (RAS) plays an important role in the pathogenesis and development of hypertension. Angiotensin converting enzyme inhibitors (ACEis) and angiotensin receptor blockers (ARBs) are commonly used in hypertensive patients as two targeted RAS system inhibitors (Ma, J., et al., 2021). DN is known to be one of the most common and serious complications of diabetes, for which there is still no adequate drug therapy. Given the importance of RAS in the pathophysiology of DN, it is thought that impaired renal function and impaired renal structure (which are associated with the progression of diabetes) may be ameliorated by blockade of RAS by ACEi or ARBs. ACEi and ARBs are the first-line medication therapies for diabetic patients with proteinuria. Our findings support that ACEi are relatively renoprotective and safe treatments as compared with ARBs in diabetic patients with proteinuria (Hsu, F.Y., и сор., 2017). Perindopril belongs to the ACEi class of medicines. It is used to treat essential hypertension (high blood pressure), heart failure, and stable coronary artery disease. The angiotensin-converting enzyme is inhibited (Hodzic, E., et al., 2020). Ang II receptor blockers, also known as angiotensin receptor antagonists, or sartans, are a group of drugs that work by modulating RAAS, blocking the action of the powerful vasoconstrictor Ang II (Scheinman, S.B., et al., 2021). Streptozotocin (STZ) is an antibiotic that destroys pancreatic islet cells and is commonly used in research to create a type 1 diabetes model (T1DM). STZ is a highly selective pancreatic islet-cell cytotoxic drug that, when given in a single large dose, causes full -cell necrosis and diabetes within 48 hours. The T1DM animals can develop diabetic complications, e.g., diabetic neuropathy, diabetic nephropathy and diabetic atherosclerosis (Furman, B. L.,2021).

The goal of our research was to see if the effects of perindopril and candesartan on serotonin levels in serum and brain tissue in a white laboratory rat with induced DN, both alone and in combination.

MATERIAL AND METHODS

1. Experimental animal

To carry out the experiments provided in this study, 125 male and female normal blood pressure white Wistar varieties of experimental rats were selected. To minimize the effects of individual differences, all animals were 9-11 weeks old and weighed about the same as 200-300 grams. The effects of external factors on renal function were minimized by the same volume of injection as standardized animal care. Of the administration solution. Rats were housed in 5 cages and were given standard laboratory rat feed and water freely.

2. Experimental model

In experimental scientific studies, rat-induced diabetes, and therefore the most used model of DN, is the induction of diabetes with a single dose of STZ.

3. How to measure serum serotonin in white laboratory rats

The enzyme-linked immunosorbent assay (ELISA) method was used to measure serotonin in the serum and brain tissue of white laboratory rats.

4. Test protocol

To respond to selected rats, they were divided into 5 groups, each consisting of 25 animals.

- Experimental animal

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• Protocol No. 2 (experimental)

This group of animals was a positive (diabetes) control group. In order to examine the symptoms and signs of DN in this group of rats, they were given saline in the same amount as the animals given the therapeutic active medication for the next 8 weeks after receiving STZ.

• No. 3 experimental protocol

Perindopril was given orally with an intragastric tube, dissolved in 5% glucose, at a dose of 6 mg / Kg / TT / day for 8 weeks to assess the therapeutic impact of ACEi perindopril monotherapy in the treatment of experimentally produced DN in this group of rats following 4 weeks of STZ administration.

• 4. Test protocol

After 4 weeks of STZ administration, oral administration of candesartan was started with an intragastric tube, dissolved in 5% glucose dose of 5mg / Kg / TT / day for 8 weeks in this group of rats to evaluate the therapeutic effect of monotherapy with AT1 - candesartan blocker in the treatment of experimentally induced DN.

• No. 5 experimental protocol

To assess the therapeutic effect and tolerability of RAS with ACE inhibitor and AT1-blocker, ie double blockade with perindopril and candesartan in the treatment of induced DN, this group of rats received perindopril and candesartan orally via an intragastric tube after 4 weeks of STZ administration. These medications were dissolved in 5% glucose and administered in substantially lesser amounts. For 8 weeks, patients were given perindopril at a dose of 3mg / Kg / TT / day and candesartan at a dose of 2.5 mg / Kg / day. The two medications were dosed separately, with perindopril being given first and candesartan being given 30 minutes later.

RESULTS

a) Results of serotonin level in serum

In the experiment, the effects of both drugs (peridopril and candesartan, given individually and in combination) on serum serotonin levels were examined. In order to take into account the possibility of interaction (positive or negative) between the two drugs, four experimental groups were formed in a 2x2 design scheme and the results were processed using the Two-Way Factorial ANOVA for Independent Samples. The statistical package SPSS 11 was used.

Scheme of design of experimental groups:

Scheme No. 1: Design of experimental groups

		Perindopril	
	Dose: 0 = without drug 1 = animals treated with drug	0	1
Candesartan	0	Control group	Treated with Perindopril
	1	Treated with Candesartan	Treated with Perindopril and Candesartan

The graph below (Figure 1) shows the mean values for the serum serotonin concentration in the four experimental groups.

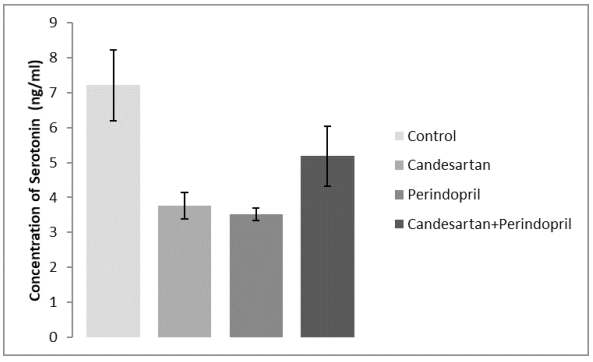

Figure 1. Serum serotonin concentration (mean ± standard error). The legend is shown on the graph itself.

From the graph shown, we can get a general impression that drug treatment leads to a decrease in serum serotonin levels. In order to make statistical processing of the data, the averages of the groups were organized in a 2x2 scheme (according to the general design given above):

Scheme No. 2: Averages of drug groups in serum

		Perindopril		
	Dose: 0 = without drug 1 = animals treated with drug	0	1	Row average:
Candesartan	0	7,209 ± 1,03	3,513 ± 0,178	5,625 ± 0,93
	1	3,766 ± 0,39	3,881 ± 0,26	3,823 ± 0,22
	Average column:	5,489 ± 0,83	3,723 ± 0,16	

The following graphs for the averages of the experimental groups were created for a clearer understanding (Figure 2):

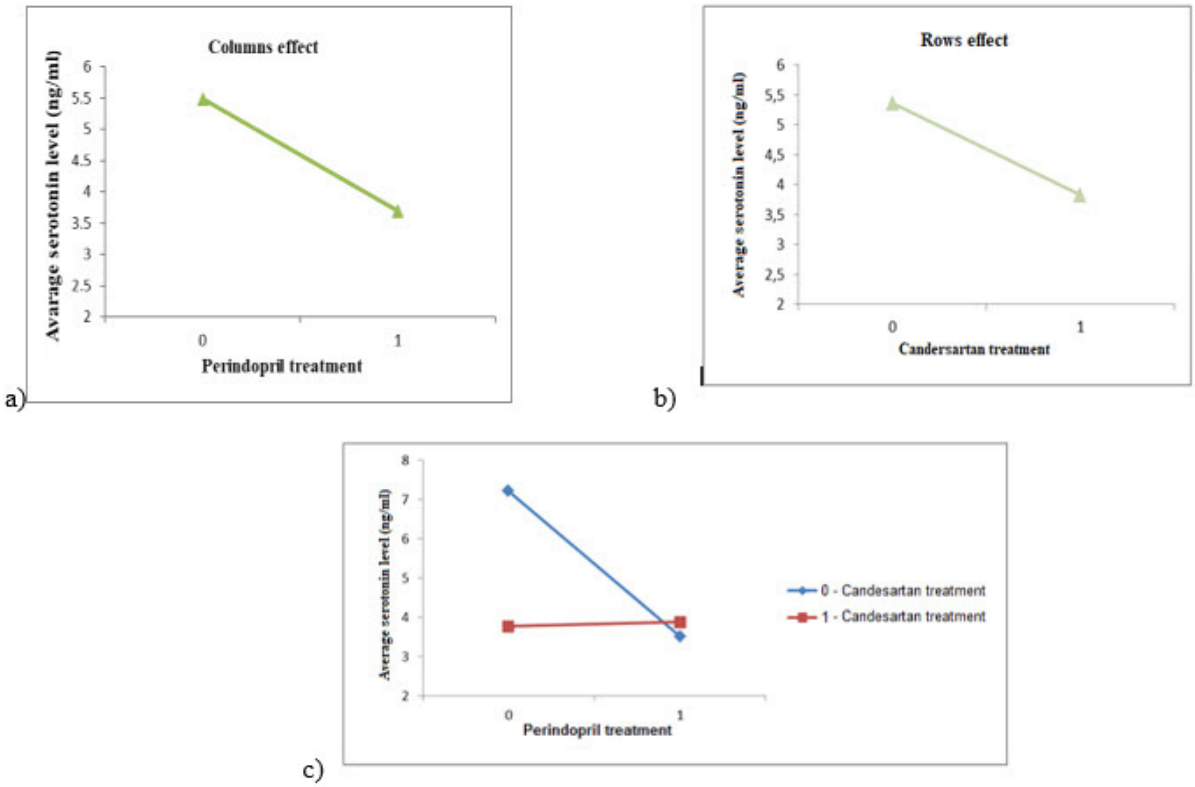

Figure 2. a) The effect of perindopril treatment (0 = control animals; 1 = treated animals). b) The effect of candesartan medication (0 = control animals; 1 = treated animals). c). Comparative graph (interaction effect) of the averages of the four experimental groups.

The intersection of the two lines (one for each of the drugs), which connect the averages when no treatment was given (0) and when the animals were treated (1), indicates the existence of a degree of interaction between the two drugs. This is confirmed by the results of the ANOVA analysis, given in the following table:

Table No. 1. Results from Two-Way Anova analysis of serum serotonin concentration data

ANOVA Summary					
Source	**SS**	**df**	**MS**	**F**	**P**
Rows	12.12	1	12.12	8.64	0.0135
Columns	11.62	1	11.62	8.28	0.015
r x c	11.83	1	11.83	8.43	0.0144
Error	15.43	11	1.4		
Total	51	14			

The influence on the columns statistics (p) truly represent the effect of perindopril medication on the level of the parameter being studied. It may be inferred that perindopril medication causes a substantial decrease in serum serotonin levels (p0.05).

The influence of candesartan medication on the level of the examined parameter is represented by the statistics (p) obtained for the effect in the rows. It may be inferred that candesartan medication causes a substantial decrease in serum serotonin levels (p0.05).

The results of the Two-Way ANOVA analysis show a significant effect of interaction between the two drugs (p <0.05). Analyzing the picture in Figure 2, it is clear that the effects of both drugs on serum serotonin levels are not simply additive. Treatment with a combination of perindopril and candesartan reduces serotonin levels to an average of 3.881 ng / ml. Animals receiving candesartan alone or perindopril alone showed even lower serum serotonin concentrations (3,766 and 3,513 ng / ml, respectively). This does not lead to the conclusion that there is a degree of negative interaction between the two drugs in terms of their effect on serum serotonin levels.

b) Results of serotonin level from brain tissue

The graph below shows the mean values for serotonin concentration in brain tissue in the four experimental groups.

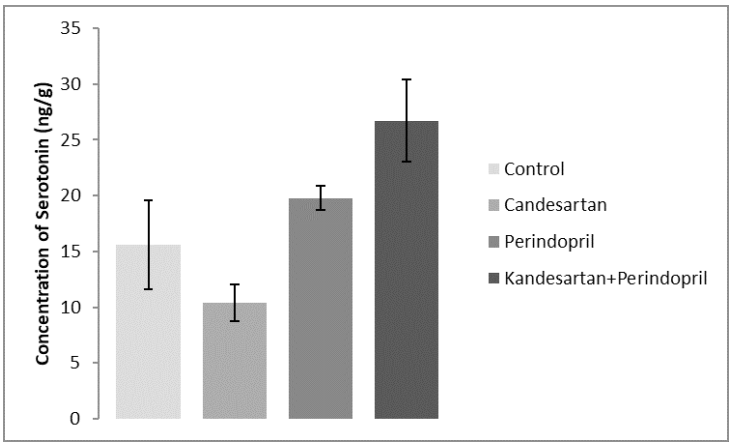

Figure 3. Serotonin concentration in the brain (mean ± standard error). The legend is shown on the graph itself.

In general, drug treatment (especially the combination of both drugs) leads to an increase in serotonin levels in the brain tissue. In order to make statistical data processing, the averages of the groups were organized in a 2x2 scheme (according to the general design given above): .

Scheme No. 3. Averages of drug groups in brain tissue

		Perindopril		
	Dose: 0 = without drug 1 = animals treated with drug	0	1	Row average:
Candesartan	0	15,595 ± 2,78	20,466 ± 0,81	17,757 ± 1,73
	1	23,178 ± 2,06	26,722 ± 3,69	24,753 ± 1,96
	Average column:	19,384 ± 2,06	23,594 ± 2,11	

For a clearer view, the following graphs for the averages of the experimental groups are constructed:

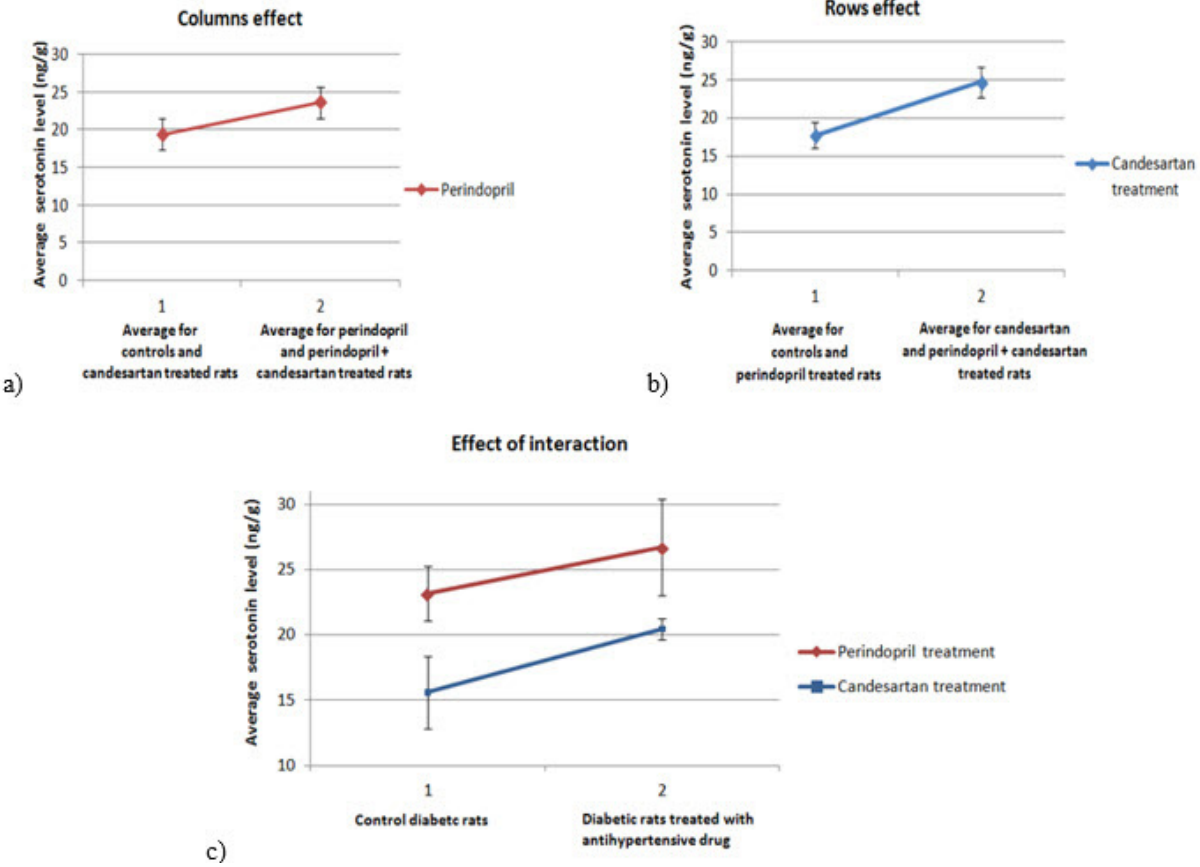

Figure 4. a) Effect of perindopril treatment (0 = level in control animals; 1 = level in treated animals). b) Effect of candesartan treatment (0 = level in control animals; 1 = level in treated animals). c) Comparative graphic (interaction effect) of the averages of the four experimental groups.

The two lines in the graph above (one for each drug), which link the averages when no treatment was given (0) and when the animals were treated (1), are almost parallel. This leads to the conclusion that there is no interaction between the two drugs. This is confirmed by the results of the ANOVA analysis, given in the following table:

Table 2. Results from Two-Way Anova analysis of data on serotonin concentration in brain tissue.

ANOVA Summary					
Source	**SS**	**df**	**MS**	**F**	**P**
Rows	220.26	1	220.26	7.51	0.0159
Columns	78.77	1	78.77	2.69	0.1232
r x c	1.97	1	1.97	0.07	0.7952
Error	410.6	14	29.33		
Total	711.6	17			

The statistics (p) for the effect in the columns represent the influence of perindopril medication on the level of the parameter under investigation. Although perindopril administration causes an increase in serotonin control levels (15,595 ng/g) in drug-treated mice, the difference in means is not statistically significant (p = 0.1232).

The influence of candesartan medication on the level of the examined parameter is represented by the statistics (p) obtained for the effect in the rows. It can be inferred that candesartan medication increases serotonin levels in brain tissue significantly (p0.05).

The results of the Two-Way ANOVA analysis show the absence of a significant interaction effect between the two drugs (p = 0.7952). Analyzing the picture in Figure 4, it is clear that the effects of both drugs on serotonin levels in brain tissue are simply additive.

Based on the absorbents obtained by measuring the prepared samples, a series of data was obtained which were statistically processed and presented graphically accordingly. In all statistical tests the level of significance is defined by p = 0.5.

DISCUSION

The goal of our study was to see if the effects of perindopril and candesartan, both separately and together, would cause substantial changes in serotonin levels in the serum of white laboratory rats with induced DN. Blood pressure and renal function are both controlled by RAAS.ARBs and ACEis have been shown to be effective in reducing hypertension, slowing the progression of diabetic and non-diabetic renal disease, reducing the risk of proteinuria, and reducing proteinuria (Burnier, at al., 2019). RAS plays an important role in the pathogenesis and development of hypertension. ACEis and ARBs are commonly used in hypertensive patients as two targeted RAS system inhibitors (Ma, J., et al., 2021). Clinical trials have shown a clinically substantial effect in reducing the course of nephropathy, especially when albuminuria is present, and worldwide guidelines suggest their usage (Leoncini, et al., 2020). Serotonin is an ancient biogenic amine that has played an important role in energy balance for billions of years, according to phylogenetic analysis. By boosting the sympathetic demand for adipose tissue, serotonin in the CNS regulates behavior, suppresses hunger, and boosts energy expenditure in mammals. In addition to these central effects, new evidence points to an important role for peripheral serotonin as a factor that improves nutrient absorption and storage. As a neurotransmitter in the CNS, it is required for several brain functions and has been linked to anxiety and behavior. Furthermore, central serotonin contributes to neuronal control of peristalsis and intestinal fluid secretion. Serotonin mediates a number of neuronal processes such as bladder function, respiratory rate, haemostasis, vascular tone, immune function, and intestinal inflammation (Yabut, J. M., et al., 2019). There is partial and/or very little confirmed information in the literature on changes in serotonin levels in a white laboratory rat with induced DN under the influence of perindopril and candesartan.

Our results showed that the blockade of Ang II AT1 receptors by candesartan significantly reduced the serum serotonin concentration, ie that the reduced effect of Ang II was associated with a decrease in serotonin. Perindopril also has a significant effect on lowering serum serotonin levels. Also, significant changes, a decrease in serum serotonin levels showed the group of animals that were treated in combination with both drugs, ie with a slight increase compared to groups of animals that were treated with drug monotherapy. These results demonstrate that dual blockade of RAS by ACEi and Ang II AT1 receptor antagonists has less effect on lowering serum serotonin levels compared to monotherapy. This does not lead to the conclusion that there is a degree of negative interaction between the two drugs in terms of their effect on serum serotonin levels. In addition, the aim of this study was to determine the potential therapeutic effect of perindopril monotherapy, candesartan monotherapy, and tolerability of combination therapy with ACEi and Ang II AT1 serotonin reuptake in the treatment of STZ-induced DN. Our results show that candesartan monotherapy causes an increase in the concentration of serotonin in the brain. In contrary, treatment with perindopril did not cause a significant change in serotonin levels in the brain. The greatest substantial impact was noticed when both medications were used at the same time, implying that, in addition to their usual antihypertensive effects, these two pharmaceuticals work as antidepressants and possible anxiolytics when used together. Our findings revealed that rat brains with a drastically diminished effect of Ang II due to double RAS blockage dramatically boosted serotonin levels, implying that lower Ang II in the brain was associated with higher serotonin levels.

CONCLUSION

During this experimental work we obtained the following conclusions:

• The results reveal that blocking the RAS with perindopril, candesartan, or their combination significantly lowered serum serotonin levels in all three groups.

• The data reveal that blocking the RAS with perindopril, candesartan, or a combination of the two dramatically boosted serotonin levels in the brain in all three groups.

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