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THE EFFECTS OF 5,5'-BUTANE-1,4-DIYLBIS{2-[(4-BENZYLPIPERAZIN-1 YL)METHYL]-4-ETHYL-2,4-DIHYDRO-3H- 1,2,4-TRIAZOLE-3-THIONE} ON MDA LEVEL AND VITAMINS IN SERUM, LIVER AND KIDNEY OF RATS

ABSTRACT

The 1,2,4-triazole and its derivatives were reported to exhibit various pharmacological activities. Mannich bases possess potent biological advantages due to being antibacterial, antifungal, anti-inflammatory, antimalarial and. They also have pesticide properties. As previously reported, Mannich bases derivatives were synthesized [1]. In this study, the effects of newly synthesized a Mannich base compound which contain bis-1,2,4- triazole on the level of malondialdehyde (MDA) and antioxidant vitamins (A,E,C) of the serum, liver and kidney of rats have been investigated. Besides, the level of malondialdehyde and vitamins has been determined by HPLC. Compared to the control group, the compound showed satisfactory performance.

Keywords: 1,2,4- Triazole, Mannich Bases, Vitamin, Serum, Kidney, Liver, Rat

5,5'-BUTANE-1,4-DIYLBIS{2-[(4-BENZYLPIPERAZIN-1YL)METHYL]-4-ETHYL-2,4-DIHYDRO-3H- 1,2,4-TRIAZOLE-3-THIONE} MANNICH BAZININ FARELERİN BÖBREK, KARACİĞER VE SERUMLARINDAKİ MDA VE VİTAMİN DÜZEYLERİNE ETKİSİ

ÖZET

1,2,4-triazol ve türevlerinin çeşitli farmakolojik aktivite gösterdiği bildirilmiştir. Mannich bazlar antibakteriyel, anti-enflamatuar, antifungal, sıtma ve pestisit özellikleri gibi güçlü biyolojik aktivitelere sahiptir. Mannich bazları türevleri daha önce bildirildiği gibi yeniden sentezlendi[1]. Bu çalışmada, bis-1,2,4 – triazol içeren bir Mannich bazı bileşiğinin farelerin serum, karaciğer ve böbreklerindeki malondialdehit (MDA) ve antioksidan vitaminler (A,E,C) seviyelerine olan etkileri incelendi. Malondialdehit düzeyi ve vitaminler HPLC tarafından belirlendi. Bileşik kontrol ile karşılaştırıldığında iyi aktivite gösterdi.

Anahtar Kelimeler: 1,2,4- Triazol, Mannich Baz, Vitamin, Serum, Karaciğer, Böbrek, Fare

1. INTRODUCTION (GİRİŞ)

In recent years, heterocyclic compounds and their analogues and derivatives have been of strong interest to researchers due to their useful biological and pharmacological properties [2].

The literature review shows that 1,2,4-triazole has a wide spectrum of biological advantages. In particular, compounds having 1, 2,4-triazole nucleus are known to have an excellent anti-bacterial, anti-fungal, anti-tubercular, anti-oxidant, anti-cancer, anti-inflammatory, analgesic, anti-convulsant, anxiolytic effect [3].

A great number of literatures have shown that Mannich bases are of a strong biological nature, which can be summed up in being antibacterial, antifungal, anti-inflammatory, antimalarial and pesticide properties [1 and 5]. The 1,2,4-triazole and its derivatives were reported to exhibit various pharmacological effects 1–4. Some of the drugs present nowadays such as ribavirin, rizatriptan, alprazolam, fluconazole and itraconazole are the best examples for potent molecules possessing triazole nucleus [1, 6 and 10].

The main purpose of this study was to assess the MDA level and antioxidant vitamins (A, C and E vitamins) in liver, kidney and serum of experimental and *control group* rats then compare these with different Mannich bases, which contain bis-1,2,4- triazole groups according to the control.

2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

The 1, 2, 4-triazole, which is a heterocyclic compound with five members, contains two carbons and three nitrogens with the molecular formula of $C_2H_3N_3$. It also has two tautomeric forms. 1*H* and 4*H*-1,2,4-triazole is regarded as a pharmacologically crucial nucleus. The literature review demonstrates that 1,2,4-triazole has a wide range of biological activities. Compounds with 1,2,4-triazole nucleus are especially renowned for their distinguished antibacterial, antifungal, ant tubercular, antioxidant, anticancer, anti-inflammatory, analgesic, anticonvulsant, anxiolytic effects [5 and 6]. As a result of their beneficial biological and pharmacological characteristics, heterocyclic compounds along with their equivalents and derivatives have recently drawn a great deal of attention.

3. EXPERIMENTAL METHOD (MATERYAL VE METOD)

3.1. Animal Treatments (Hayvan Uygulamaları)

The following experiments were approved by the Ethical Committee for the care and use of laboratory animals in Firat University. Experiments were performed 4-6 times per week on male Long Evans rats, weighing 150-200g. They were allowed unrestricted access to food and water. Room temperature was maintained at 22±2°C with a 12-hour's light-dark cycle. The animals were randomly divided into two groups (the Control and L groups) with each group containing five rats. The Mannich bases, which contain bis-1,2,4-triazole derivatives were diluted with corn oil in such a manner that their amount would be below 10% as dimethylsulfoxide (DMSO) also dissolved [9].

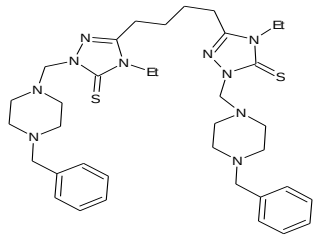
Animals were divided into one *control group* and another *implementation group,* with five rats in each group. DMSO, diluted using corn oil only, was injected to the control group. 0.5 ml DMSO including 25 mg/kg was injected subcutaneously to derivates groups for thirty days with a three-day interval throughout the test.

These procedures were repeated for thirty days after which time each experimental rat was anesthetized and decapitated and blood samples were collected in tubes and stored in -20°C prior to the biochemical analysis. Blood samples centrifuged at 4500×g for 10 minutes, and the serum was separated to get tested for vitamins and

MDA. Besides, livers and kidneys were removed for vitamins and MDA analyses. 300 mg liver, 300 mg kidney tissue samples were homogenized in 3 ml acetonitrile/methanol/isopropyl alcohol-containing (2:1:1, v/v/v) tubes and the samples were vortexed for thirty seconds and centrifuged at 6000×g for 10 minutes. Supernatants were transferred to the auto-sampler vials of the HPLC instrument [1, 11, 12, 15, 18 and 32].

3.2. Chemicals (Kimyasallar)

All solvents were of analytical-grade reagents. The Mannich bases containing bis-1,2,4-triazolederivatives, used in the applications, were synthesized and characterized by Koparır et al. [1]. The structure of derivatives and IUPAC Nomenclature are below (Fig. 1 and Tab.1).



L

Figure 1. Chemical structure of L compound
(Şekil 1. L bileşiğinin kimyasal formülü)

Table 1. IUPAC Nomenclature of compound L.
(Tablo 1. L bileşiğinin adlandırılması)

L	5,5'-Butane-1,4-diylbis{2-[(4-benzylpiperazin-1-yl)methyl]-4-ethyl-2,4-dihydro-3H- 1,2,4-triazole-3-thione}

3.3. Analytical Methods (Analitik Metotlar)

The liquid chromatographic system (Shimadzu) consisted of two LC-20AD pumps, aDGU-20A5 degasser, a Sil20A auto sampler, a CTO-10As VP column oven, an SPD-M20A DAD system, and an RF-10AXL Fld system. The two detectors were connected in series.

3.3.1. Determination of Tissues Vitamin C and MDA Levels (Dokularda C Vitamini ve MDA Düzeylerinin Belirlenmesi)

0.3 grams of liver and kidney tissue samples were taken and homogenized in a mixture of 1.5 ml of $HClO_4$ (0.5 M) and a 1.5 ml of distilled water. Following this step, tissue samples were centrifuged at 4500 rpm for 25 minutes. After the mixture centrifuged, 20 µl of samples were carefully taken from supernatants and injected into the HPLC system. The detection was performed at 254 nm for vitamin C and MDA. Results were calculated as µg/g MDA tissue and as µg/g for C vitamin [15 and 30].

3.3.2. Determination of Tissues Vitamin A and E Levels (Dokularda A ve E Vitamini Düzeylerinin Belirlenmesi)

0.3 grams of liver and kidney tissue samples were taken and 4 ml of ethyl alcohol (containing 1%H_2SO_4) were added for precipitated proteins. After vortexing, the samples were centrifuged at 4500 rpm for 25 minutes. After the mixture centrifuged, 0.3 ml of n-hexane was added and the tubes were vortexed and centrifuged once more. At the end of the centrifugation process, the hexane was taken carefully to a glass tube and 0.3 ml of n-hexane was added and centrifuged one more

time. The Hexane phase was then caused to evaporate with nitrogen flow and the residue was made to dissolve in 100 µL of methanol and 20 µl of sample were injected into the HPLC system. The detection was performed at 326 nm for vitamin A, and 296 nm for vitamin E. The results of the analysis were expressed as µg/g [15, 30 and 33].

3.3.3. Determination of Serum Vitamin C and MDA Levels (Serumda C Vitamini ve MDA Düzeylerinin Belirlenmesi)

A volume of 0.3 ml of serum sample was taken then 0.3 ml of 0.5 M $HClO_4$was added for precipitated proteins. This mixture was then vortexed by adding pure water to the total 1 ml volume. After 15 minutes, the mixture centrifuged (2500 rpm/min) and then 20 µl of samples were cautiously taken from above supernatants and were injected on the HPLC. The detection was performed at 254 nm for vitamin C and MDA. At the end of the procedure, results were calculated as µg/mL for MDA and C vitamin [16].

3.3.4. Determination of Serum Vitamin A and E Levels (Serumda A ve E Vitamini Düzeylerinin Belirlenmesi)

0.3 ml of serum samples was taken and 0.3 ml of ethyl alcohol (containing 1%H_2SO_4) was added for precipitated proteins. After vortexing, the samples were centrifuged at 2500 rpm for 5 minutes. When the mixture finally centrifuged, 250 µl of n-hexane were added and the tubes were vortexed and centrifuged once again. At the end of the centrifugation process, the hexane was gently taken to a glass tube and 250 µl of n-hexane were added and centrifuged one more time. Similar to the previous steps, the Hexane was caused to evaporate with nitrogen flow and the residue was made to dissolve in a 100 µl of methanol and 20 µl of sample were injected into the HPLC system. The detection was performed at 326 nm for vitamin A, and 296 nm for vitamin E. Results were calculated as µg/mL for A and E vitamins [17, 18, 30 and 33].

3.4. Statistical Analysis (İstatistiksel Analizler)

For a statistical analysis, the SPSS 15.0 software program was utilized. The experimental results were reported as mean ± S.D. The comparison between the experimental group and the control one was drawn, using ANOVA and LSD tests.

4. FINDINGS (BULGULAR)

4.1. Tissues Vitamin A,E,C and MDA Levels (Dokularda Vitamin A,E,C ve MDA Düzeyleri)

All results of liver tissues are presented in Table 2.

Table 2. The contents of vitamins (A,C,E) and MDA levels in the liver tissues of the rats belonging to both the experimental and control groups
(Tablo 2. Deney ve kontrol grubu rat karaciğer dokularında vitamin A,E,C ve MDA düzeyleri)

Parameters	Groups	
	Control	L
C Vitamin (µg/g)	20,72±1,47[a]	21.10±0.84[a]
MDA (µg/g)	4,96±1,08	3.10±0.31
A Vitamin (µg/g)	0,24±0,01[a]	0.23±0.01[a]
E Vitamin (µg/g)	0,55±0,06[ab]	0.57±0.01[ab]

a-b Mean values with different superscripts found on the same row are significantly different. *P<0.05; Nİ; unimportant p>0.05, P: Statistical values

Vitamin C level significantly increased in the liver tissue in the L-treated groups when compared to the control group. However, Vitamin A level was similar in the L-treated group when compared to the control group, while vitamin E level increased in the L-treated group. MDA level decreased in both the L and control groups and statistical differences were shown, compared to control group.

Table 3. The contents of vitamins (A,C,E) and MDA levels in the kidney tissues of the rats belonging to both the experimental and control groups
(Tablo 3. Deney ve control grubu rat böbrek dokularında vitamin A, E, C ve MDA düzeyleri)

Parameters	Groups	
	Control	L
C Vitamin (µg/g)	9,26±1,09	8.76±0.29
MDA (µg/g)	2,90±0,05^{ab}	2.44±0.16^{b}
A Vitamin (µg/g)	0,24±0,02^{a}	0.23±0.05^{a}
E Vitamin (µg/g)	0,74±0,06^{b}	0.85±0.03^{ab}

a-b Mean values with different superscripts on the same row are significantly different.
*P<0.05; Nİ; unimportant p>0.05, P: Statistical values

Vitamin C level decreased in the kidney tissues in the L-treated group and statistical differences were shown when compared to control group. Vitamin A level was similar in the L-treated groups, but not when compared to the control group. Vitamin E level increased at the L-group, as well as when compared to the control one. The MDA level decreased in the L-treated group when compared to the control one.

4.2. Serum Samples Vitamin A, E, C and MDA Levels

All results are shown in Table 4.

Table 4. The contents of vitamins (A,C,E) and MDA levels in the serum of the rats of both the experimental and control groups
(Tablo 4. Deney ve kontrol grubu rat serumda vitamin A,E,C ve MDA düzeyleri)

Parameters (µg /mL)	Groups	
	Control	L
C Vitamin	4,00±0,19^{a}	4.36±0.15^{ab}
MDA	0,57±0,02^{a}	0.49±0.03^{b}
A Vitamin	0,57±0,02^{a}	0.59±0.02^{a}
E Vitamin	3,34±0,20^{ab}	3.72±0.05^{ab}

a-b Mean values with different superscripts on the same row are significantly different.
*P<0.05; Nİ; unimportant p>0.05, P: Statistical values

In the serum samples, vitamin A, and C level was of similar values in the L-treated group when compared to the control one. The level of vitamin E significantly increased in the L-group than in the control group. Also, MDA level decreased in the L-treatment group than in the control one.

5. CONCLUSION AND RECOMMENDATIONS (SONUÇLAR VE ÖNERİLER)

Reactive oxygen species, particularly free radically induced lipid peroxidative tissue damage, have been implicated in the pathogenesis of various diseases. Lipid peroxidation is assessed in an indirect manner by the measurement of secondary products, such as malondialdehyde (MDA) [18 and 28]. MDA is a three-carbon, low molecular weight aldehyde and a spontaneous, breakdown product of peroxides, which can be produced from free radical attack on poly unsaturated fatty acids [17 and 20]. The determination of MDA is one

of the most commonly used methods for monitoring lipid peroxidation [18, 20 and 29]. Several methods are available for the quantification of MDA in biological samples. Despite being simple and reproducible, the frequently used TBA method is fairly sensitive, however, not specific [20, 21 and 29]. Recently, various HPLC methods have been applied to improve the specificity of the MDA method [20, 25 and 29].

Vitamin A has multiple functions: Firstly, it is important for growth and development, as well as the maintenance of the immune system and good vision [26]. Both vitamin E and C react rapidly with organic free radicals, and it is widely accepted that the antioxidant properties of these compounds are partially responsible for their biological activity. Nevertheless, vitamin E is considered more lipophilic than vitamin C, and it has been found to be the most potent antioxidant in bio-membranes, particularly with respect to lipid peroxidation. Penetration into a precise site in the membrane is probably a significant characteristic of the protection against highly reactive radicals [28, 31 and 32].

In this study, MDA and antioxidant vitamins were both detected in the serum and the liver and kidney tissues obtained from healthy rat tissues by HPLC. According of our study results for liver and kidney tissues and in serum, compounds L reduced the MDA level compared to the control group. Also the L-compound increased vitamin C level and showed closely similar vitamin A and E levels compared to the control group. The results concluded that compounds L, with benzylpiperazine moiety, exhibited the best biological effects. Thus, it was concluded that the presence of the benzylpiperazine moiety was found to be highly essential for effective biological activity. The structure and biological activity relationship of title compound showed that the presence of biologically active groups such as benzylpiperazine group, attached to the triazole ring of the title compounds, is responsible for satisfactory antioxidant activity. We suggested that it is useful to use medicines that contain piperazine moieties

In conclusion, the result study reports the successful biological activity of a new Mannich base compound, which contains bis-1,2,4-triazole groups. The antioxidant activity revealed that the tested compound has good antioxidant effects, which may be due to the presence of piperazine moiety. Hence, it is concluded that there is ample scope for further study.

REFERENCES (KAYNAKLAR)

1. Koparır, M., (2013). Synthesis and Biological Activities of Some New Mannich Bases of 5,5_-Butane-1,4-diylbis[4-ethyl-2,4-dihydro-3H-1,2,4-triazole-3-thiones. Chem Sci Trans., 2(3), 701-710, http://www.e-journals.in/PDF/V2N3/701-710.pdf (2015).
2. Hazra, K.G., Nargund, L.V., Rashmi, P., Chandraa, J.N., Narendra, S., and Nandha, B,. (2011). Synthesis of Some Novel 2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazole-3,3-dioxideby [4+2] Diels-Alder Cycloaddition Reaction as Antioxidant Agents, Journal of Pharmacy Research.
3. Wahi, A.K. and Singh, A., (2011). Triazole: Recent development and biological activities. Asian Journal of Biochemical and Pharmaceutical Research; 1(2):193-205.
4. Legrand, C., Ropaa, P., Vulturescu, B., Hogeac, A.M., Surpateanu, G.G., Cazier, F., Woisel, P., and Surpateanu, G., (2005). Progress in Organic Coatings, 53, 106-111.
5. Ewiss, N.F., Bahajaj, A.A., and Elsherbini, E.A., (1986). J Heterocycl Chem., 23, 1451-1458.

6. Eweiss, N.F. and Bahajaj, A.A., (1987). J. Heterocycl Chem., 24, 1173.
7. Prasad, A., Ramalingam, R.J., Rao, A.B., Diwan, P.V., and Sattur, P.B., (1989). Eur. J. Med. Chem., 24, 199-201.
8. Lopes, F., Capela, R., Goncaves, J.O., Horton, P.N., Hursthouse, M.B., Iley, J., Casimiro, C.M., Bom, J., and Moreire, R., (2004). Tetrahedron Lett, 45, 7663-7666.
9. Sriram, D., Bal, T.R., and Yogeesswari, P., (2005). Med. Chem. Res., 14, 11-28.
10. Knabe, J., Buch, H.P., and Schmitt, W., (1983). Arch Pharm Chem Life Sci., (316, 1051-1053.
11. Varvaresou, A., Tsantili-Kakoulidou, A., Siatra-Papastaikoudi, T., et al., (2000). Arzneimittel for schung 50, 48.
12. Cesur, N., Birteksöz, S. ve Ötük, G., (2002). Acta Pharm Turcica, 44, 23.
13. Yue, K.K., (2003). Redox changes precede the occurrence of oxidative stress in eyes and aorta, but not in kidneys of diabetic rats, Life Sciences, 1003.
14. Karahan, I., (2010). "The relations of antioxidant vitamins A, E, and C with MDA levels in serum and liver of rats treated with nitrosamines", Toxicological and Environmental Chemistry.
15. Cetinkaya, N. ve Ozcan, H., (1991). Comp Biochem Physiol A Comp Physiol; 8,100:1003.
16. Karatepe, M., (2004). LC-GC North America. 22,362-5.
17. Catignani, G.L., (1983). Clin. Chem., 2914, 708-712.
18. Henning, S.M., Swendseid, M.E., Ivandic, B.T., and Liao, F., (1997). Free Radic. Biol.Med., 23(6):936-42.
19. Anoopkumar-Dukie, S., Walker, RB., and Daya, S.A., (2001). J Pharm Pharmacol; 53: 263-6.
20. Cordis, GA., Das, D.K., Riedel, W., (1998). J Chromatogr A; 798: 117-23.
21. Templar, J., Kon, S.P., Milligan, T.P., Newman, D.J., and Raftery M.J., (1999). Neprol Dial Transplant; 14: 946-51.
22. Slatter, D.A., Bolton, C.H., and Diabetologia, A.J., (2000). 43: 550-7.
23. Pilz, J., Meineke, I., and Gleiter, C.H., (2000). J Chromatogr B Biomed Sci Appl; 742: 315-25.
24. Dib, M., Garrel, C., Favier, A., Robin, V., and Desnuelle, C., (2002). J Neurol; 249: 367-74.
25. Verbunt, R.J. and Egas, J.M., (1996). A Van der Laarse. Cardiovasc Res; 31: 603-6,
26. Albers, D.S., Augood, S.J., Martin, D.M., Standaert, D.G., Vonsatte, J.P., and Beal, M.F., (1999). J Neurochem; 73: 881-4.
27. Tanumihardjo, S.A., (2011). The American Journal of Clinical Nutrition 94 (2): 658S–665S.
28. Slater, T.F., (1978). In Biochemical Mechanisms of Liver Injury 745–801.
29. Tüközkan, N., Erdamar, H. ve Seven, I., (2006). Measurement of Total Malondialdehyde in Plasma and Tissues by High-Performance Liquid Chromatography and Thiobarbituric Acid Assay 11, Sayı 2, 088-092,http://www.firattipdergisi.com/text.php3?id=334 (2015).
30. Turkoglu, S., Parlak, A.E., Yildirim, I., Karagozoglu, Y. and Alayunt, N.O., (2014). The effects hydroxyurea derivative schiff bases on fatty acids and lypophilic vitamins in liver of rats, D.J. of N. and B. Vol. 9, No. 1, 159–165.
31. Packer, J.E., Slater, T.F., and Willson, R.L., (1979). Direct observation of a free radical interaction between vitamin E and vitamin CNature 278, 737-738 doi:10.1038/278737a0

http://www.nature.com/nature/journal/v278/n5706/abs/278737a0.html(2015).
32. Karagozoglu, Y., Turkoglu, S., Parlak, A.E. ve Alayunt, N.O., (2013). The effects of hydroxyurea derivative 1, 3, 4-thiadiazoles on serum biochemical parameters and antioxidant status in liver of rats digest journal of nanomaterials and biostructures vol. 8, no. 1, 247 - 253 http://www.chalcogen.ro/247_Turkoglu.pdf (2015).
33. Keser, S., Erman, O., Yılmaz, O., Irtegun, S., and Tuzcu, M., (2012). Effects of antioxidants resveratrol, catechin and lipoic acid and carcinogen kbro3 on lipophylic vitamins and cholesterol in lung, liver and kidney of wistar rats, Kafkas Univ. Vet. Fak. Dergisi, 18 (3), 367-372.