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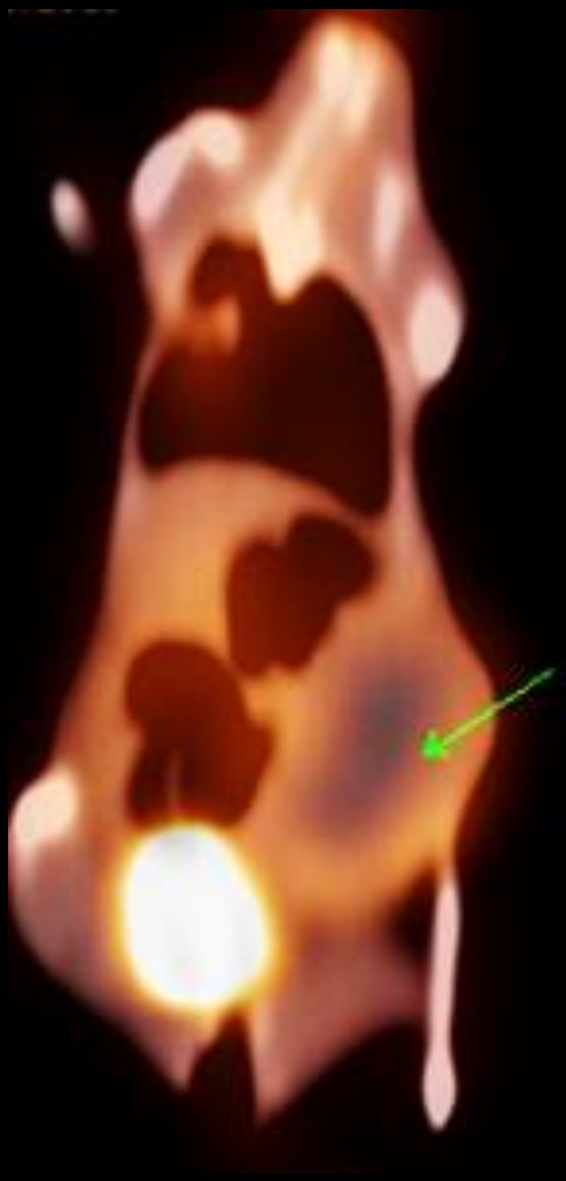

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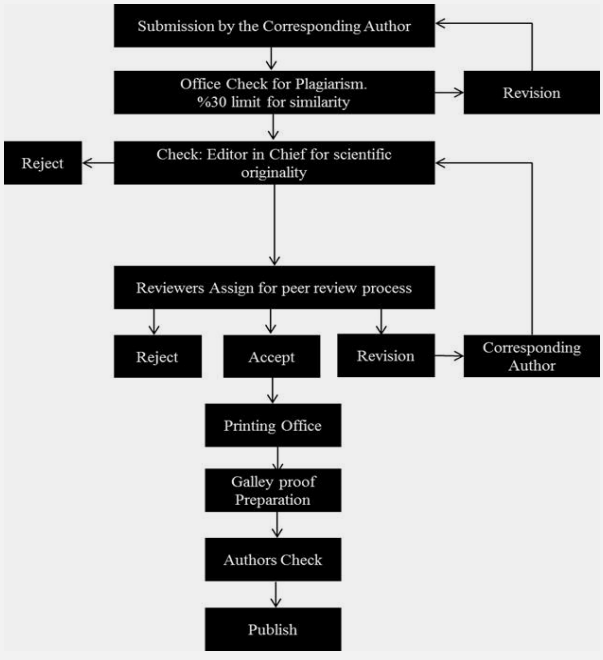

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Investigation of antioxidant and antimicrobial activities of medicinal plants grown in the Eastern Blacksea region of Turkey

Sule Ceylan[1]*, Burhan Harsit[2], Ozlem Saral[3], Mehmet Ozcan[4], Emine Sonmez[5]

Abstract

Objective: The aim of this study was to screen various extracts of plant of Gentian (*Gentiana pyrenaica* L.), Tarragon (*Artemisia dracunculus* L.), Persimmon (*Diospyros kaki*), Raspberry (*Rubus ideaus*) to display potent antimicrobial, antifungal and antioxidant activity in vitro, total phenolic and flavonoid contents in order to find possible sources for future novel antioxidants in food and pharmaceutical formulations.

Material and Methods: The antioxidant properties of 12 different samples of medicinal and aromatic plants such as leaves, flowers and scapus were investigated by DPPH, FRAP and CUPRAC assays. Total phenolic, total flavonoid content and the antimicrobial properties of extracts from these plants were also determined. Antibacterial and antifungal activities were investigated by microdilution method and agar diffusion method respectively.

Results: According to antioxidant results, dried leaves of Persimmon (*Diospyros kaki*) (obtained from Trabzon) plant had the best antioxidant activity that was carried out in all analyzes (except the analysis of total polyphenol). In accordance with analysis of total polyphenol, activity of purple flower of Gentian (*Gentiana pyrenaica* L.) plant was measured at 31,303±0,274 mg GAE /g dry sample and thus this plant had the highest total phenolic content. Antimicrobial activity tests were carried out by using disc diffusion methods with 12 microbial species and most of them displayed good-moderate antimicrobial activity.

Conclusion: Due to their antimicrobial, antifungal and antioxidant properties, the extracts some of these plants might be used as potential sources of natural antioxidant and antimicrobial agents.

Keywords: Gentian, Persimmon, Raspberry, Tarragon, Antioxidant, Antimicrobial

Introduction

Phenolic compounds are known as common plant secondary metabolites that have physiological functions in plants and positive effects for human health because they can act as antioxidants (1,2). Free radical scavengers/Antioxidants have vital effects on preventing chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases and can enhance immune function. Antioxidant defenses protect the body from the harmful effects of free radicals produced by products of normal metabolism (3). Besides antioxidative properties, it was reported that phenolic compounds obtained from different plants had antimicrobial activities against different pathogenic microorganisms in literature (4–6).

Interest in medicinal plants as an alternative to synthetic drugs, especially against microbial agents owing to the development of antibiotic resistance, is increasing day by day (7). Thus, the need of finding new antimicrobial agents like phenolic compounds has become crucial. Medicinal plants are commonly used in daily life as a part of traditional remedies in Turkey. The flora in Turkey has a large variety and it is a good source for medicinal plants (8).

According to the investigations of WHO, approximately 9.000 species of 20.000 plants used for medicinal purposes in the world have been recorded from the flora of Turkey (9).

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Medicinal plants provide a wider source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained (10). Plants can product certain bioactive molecules, such as phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (11). These constituents with phenolic structures can inhibit bacterial or fungal growth (12).

The genus Gentiana comprising 400 species, is mainly distributed in Southeast Asia, Europe and North America. Some Gentiana species have been employed as folk medicine since ancient times. The underground parts of various Gentiana species have been included in many herbal formulations as remedies for poor appetite, digestive problems and as hepatoprotective agents worldwide. As natural sources of food flavoring they are utilized in alcoholic and nonalcoholic beverages (13).

Artemisia dracunculus L. (Tarragon) is an important species in Artemisia genus and has approximately 800 species which are widely distributed throughout the world.Artemisia genus is industrially important due to its antifungal, insecticidal, allelopathic, antibacterial, and other characteristics. Furthermore, the plant is useful in Unani, Homeopathy, Ayurveda, and Siddha (14)

Diospyros kaki is the edible fruit of the persimmon tree which belongs to Ebenaceae family. The fruit is a seasonal fruit with important health benefits and consists of a berry, as large as an apple, orange in colour, with soft, sweet when it ripens. The persimmon tree (Diospyros kaki) is used in traditional medicine to treat apoplexy, arteriosclerosis, cough, and diarrhea. Many studies have addressed the antibacterial, antifeedant, antifungal, anti-dust miticidal, antimalarial, and cytotoxical activities of D. kaki root-derived materials (15).

Blackberries, raspberries (Rubus ideaus) and other small fruits are an excellent source of natural antioxidants, which is one of the major reasons for their increasing popularity in the human diet. Most of these fruits belong to the diverse Rubus genus, which consists of 250 species. Many Rubus fruits are consumed fresh or as processed products such as jams, jellies, syrups and wines. The leaves and roots have been used in various medicinal applications (16).

Medicinal plant species represent a large source of new compounds that help for the preparation of new drugs. The therapeutic activity of plants is due to their biologically active polyphenolic compounds. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential. The purpose of the present study was to investigate the antioxidant and antimicrobial properties of 12 different extracts of non-wood forest products, such as leaves, flowers, fruits, roots and scapus of some species of Gentian (Gentiana pyrenaica L.), Tarragon (Artemisia dracunculus L.), Persimmon (Diospyros kaki), Raspberry (Rubus ideaus) plant extracts used for medical purposes in the Eastern Anatolia Region of Turkey (Artvin, Trabzon and Bayburt).

Materials and methods

1. The Chemicals

Methanol, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu's phenol reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Sodium carbonate, acetic acid, neocuproine (2,9–dimethyl-1,10-phenanthroline), aluminium nitrate nonahydrate and ammonium acetate were purchased from Merck Chemical Co. (Darmstadt, Germany). The chemicals were analytical degree.

2. The Plant Material

Gentiana pyrenaica L. was collected in Murgul-Tiryal, *Artemisia dracunculus* L. was collected in Bayburt-Demirözü, *Diospyros kaki* and *Rubus ideaus* were collected from two different regions (Artvin-Hatila, Trabzon-Yeniköy). Collected plant materials were dried in the oven at 40°C before treatments. Approximately 10 g of dried sample of the fruits was used to prepare methanolic extracts for each species. These preparations were used to determine antioxidant activities, and the treatments were done three times. Spectrophotometric methods were used on total polyphenols, total flavonoids and antioxidant tests. Spectrophotometric methods are frequently used for the standardization of natural raw materials.

2.3. Total Phenolic Assay

The total phenolic content of plants has been determined by using the Folin-Ciocalteu assay (17). In this study, gallic acid (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/ml) was used as a standard. Briefly, 20 µL of various concentrations of gallic acid and 20 µL methanolic samples (1 mg/ml), 400 µL of 0.5 N Folin-Ciocalteu reagents and 680 µL of distilled water were mixed and the mixture was vortexed. Following 3-minute incubation, 400 µL of Na_2CO_3 (10%) solution was added and after the process of vortexing, the mixture was incubated for 2 hours. After the incubation period at the room temperature, absorbances of the mixtures were measured at 760 nm. The concentrations of total phenolic compounds were calculated as mg of gallic acid equivalents per g of the dry weight of samples.

2.4. Total Flavonoid Assay

The total flavonoid content was measured by using the aluminum chloride assay (18). Quercetin was used as a standard. 0.5 ml of Quercetin (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/ml), 4.3 ml methanol 0.1 ml 10% $Al(NO_3)_3$ and 0.1 ml 1 M NH_4CH_3COO were added in the test tubes and then they were mixed. Mixtures were incubated for 40 minutes. After incubation, absorbance was measured at 415 nm. The total flavonoid contents of plants were expressed as mg quercetin equivalents per g of dry weight sample.

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2.5. The Determination of Antioxidant Activity

The antioxidant activities of the samples were determined using by The ferric reducing ability of plasma (FRAP), cupric reducing antioxidant capacity (CUPRAC) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay methods. The FRAP method was used for the determination of total antioxidant capacity, based on the reduction of yellow Fe^{3+} -TPTZ complex to the blue Fe^{2+} -TPTZ complex by electron donating substance under acidic condition (19). The 3 ml of FRAP reagent (containing TPTZ, $FeCl_3$, and acetate buffer) and 100 µL of the test sample or the blank (solvents used for extraction) were added to the test tube and mixed. Maximum absorbance values at 593 nm were recorded for 4 min at 25°C. The final absorbance was compared with the standard curve (100-1000 µmol/L). The data were expressed as µmol $FeSO_4.7H_2O$ equivalents per gram of dry matter.

The CUPRAC method is comprised of mixing the antioxidant solution (directly or after acid hydrolysis) with a copper (II) chloride solution, a neocuproine alcoholic solution, and an ammonium acetate aqueous buffer at pH 7, and subsequently measuring the developed absorbance at 450 nm after 60 minutes (20). 1ml 10 mM $CuCl_2$, 1ml 7.5 mM Neocuproine and 1ml 1M NH_4Ac were added test tubes, than 0.2 ml sample and 0.9 ml H2O added and mixed. End volume was 4.1 ml. Then, the final absorbance was measured at 450 nm. The test results were evaluated by Trolox ® equivalent antioxidant capacity (TEAC).

The radical scavenging activity of methanolic extracts against 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical was measured at 517 nm in spectrophometer. The assay is based on the color change of the DPPH solution from purple to yellow as the radical is deactivated by the antioxidants (21). Briefly, various concentrations for 0.75 ml of each sample extracts were mixed with 0.75 ml of a 0.1 mM of DPPH in methanol.

The radical scavenging activity was measured by using Trolox as standards and the values are expressed as IC50 (mg or mg sample per ml), the concentration of the samples that causes 50% scavenging of $DPPH^{\bullet}$ radical.

6. The Biological Materials

The total of 12 bacteria strains has been used in this study (Table 1). All test microorganisms obtained from Karadeniz Technical University, Farabi Hospital, Trabzon, Turkey where the organisms were clinically isolated from patients.

The microorganisms were storaged at -80 °C in the Microbiology laboratory, Faculty of Science at the Karadeniz Technical University, Trabzon, Turkey where the antimicrobial tests were carried out. The strains were activated at 37°C for 24 h on muller hinton agar before use. The food-associated microorganisms were selected because they are frequently reported in food.

Table 1. The name and ATCC numbers of microorganisms used in the experiments

The Name	ATCC Numbers
Gram +	
Bacillus subtilis	ATCC 6633
Enterococcus faecalis	ATCC 29212
Staphylococcus aureus	ATCC 25923
Staphylococcus epidermidis	ATCC 12228
Gram -	
Escherichia coli	ATCC 25922
Klebsiella pneumonia	ATCC 13883
Pseudomonas aeruginosa	ATCC 27853
Proteus vulgaris	ATCC 13315
Salmonella typhimirium	ATCC 14028
Yersinia pseudotuberculosis	ATCC 911
Enterobacter cloaceae	ATCC 13047
Eukaryote	
Candida albicans	

7. The Antimicrobial Activity

7.1. Disc-diffusion assay

At first the antimicrobial activity of the extracts was determined by means of the disc diffusion method which is widely used for quick screening of natural products (22-24). All extracts were dissolved in solvent (methanol); the final concentration was 10 µg/disk. Cultures of each bacteria were inoculated to Muller-Hinton agar and incubated at 37° C for 16 hours, then their concentration adjusted to 0.5 McFarland standard turbidity (approximately 1 x 10^7 - 1 x 10^8 CFU/ml) with steril %0,09 isotonic solution. One hundred micro liter of each bacterial suspension was placed onto the surface of Mueller-Hinton agar in a 60-mm Petri dish and spread homogeneously with a Drigalski tip. The disc (6 mm in diameter) was embrued with extracts and placed on inoculated muller hinton agar. Negative controls were prepared using the same solvent (methanol) employed to obtain the extracts. Kanamycin were used as positive reference at 10 µg/disk (Sigma). The inoculated plates were incubated at 37 °C for 24 h for clinical bacterial strains and at 35 °C for 48 h for yeast. The inhibition zones were measured with a caliper considering the total diameters. Each experiment was performed in triplicate. The bacteria, inhibition zone in diameter ≥ 6 mm around the disks impregnated with methanol extract, were used for minimal inhibitory concentration (MIC).

7.2. Minimal inhibition concentration (MIC)

The MIC values were determined for the bacterial strains that were sensitive to the synthetic extract in the disk diffusion assay. The inoculum of the bacterial strains were prepared from 12 h agar cultures, and suspensions were adjusted to 0,5 McFarland standard turbidity. The extracts dissolved in methanol, were first diluted to the highest concentration (500 µg/ml) to be tested, and then serial 2-fold dilutions were made to obtain a concentration range from 500 µg/ml to 0,49 in 1 ml sterile test tubes containing Muller-Hinton broth.

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The MIC values of the synthetic extracts against bacterial strains were determined on the basis of a micro-well dilution method (22-24). Five hundred microliters from the stock solutions of synthetic extract prepared at the 5000 μg/ml concentration was added into the first sterile tube containing 4500 μl Muller-Hinton broth.

Then, 2500 μL from the serial dilutions was transferred into the eleven consecutive tubes. The last tube (twelve) containing 2500 µL of Muller-Hinton broth without compound.

The final volume in each tube was 2500 µL. Kanamycin at a concentration range of 500-0,49 µg/ml was prepared in Muller-Hinton broth and used as a standard drug for positive control and with the inoculum on each strip was used as a negative control. The 96-well plates were prepared by dispensing 200 µL of Muller-Hinton broth containing the diluted compound into each well, and 5 µL of 0,5 McFarland from 12 h agar cultures was added into each well.

The plate was covered with a sterile plate sealer. The contents of each well were incubated at 37°C temperatures for 24 h. The MIC was defined as the lowest concentration of extract to inhibit the growth of microorganisms. The extract tested in this study was screened twice against each organism.

Results

In general, phenolic acids and flavonoids are antioxidant molecules. High antioxidant value of these molecules indicates to high antioxidant properties of plants (25, 26). It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health benefical effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and destruction of microorganisms, insects, and herbivores (27).

The total phenolic and total flavonoid contents of plants, FRAP and CUPRAC values have been shown in Table 2.

Results showed that the highest phenolic content value obtained from G. pyr. fmt and G. pyr. rmt while D. kak. lah and D. kak. ltyshowed highest flavonoid contents. Between species the highest content of polyphenols and flavonoid were observed Gentian and Persimmon. In addition to these, D. kak. ltk and R. ide.lah showed maximum activity according to the FRAP whereas D. kak. ltk and G. pyr. rmt showed maximum activity according to the CUPRAC.

The IC50 values determined from analysis of DPPH were showed in Fig. 1.

Although D. kak. ltk and D. kak. lah had the highest DPPH radical cleaning, the lowest activity was obtained from A. dra. sbd.

Table 2. Results of phenolic contents, flavonoid contents, FRAP and CUPRAC for Gentian Tarragon, Persimmon and Raspberry species*

Samples*	Total phenolics (mg GAE/g)	Total flavonoid (mg QE/g)	FRAP (µmol Fe/g)	CUPRAC (mmol TEAC/g)
G. pyr. fmt	31.303 ±0.274	18.058±0.529	54.463±0.515	0.310 ±0.008
G. pyr. rmt	15.048 ±0.391	26.230±1.113	66.063 ±1.908	0.325 ±0.002
A. dra. lbd	2.681±0.120	10.975±0.270	18.844±1.165	0.178 ±0.012
A. dra. sbd	3.010 ±0.103	3.219±0.248	7.781±0.256	0.025 ±0.001
D. kak. fah	4.354±0.254	9.731±0.969	59.410±1.316	0.193 ± 0.051
D. kak. lah	10.989 ±1.257	84.236±2.461	89.108 ±2.609	0.251 ±0.031
D. kak. fty	2.008 ±0.045	0.457±0.053	30.064 ±0.653	0.229 ±0.050
D. kak. lty	11.182 ±1.874	64.512±4.153	115.526 ±3.932	0.559 ±0.063
R. ide. fah	6.047 ±0.615	10.975±0.270	62.289±0.466	0.193 ± 0.051
R. ide. lah	11.644±0.770	17.926±1.155	107.074±3.292	0.254 ±0.030
R. ide. fty	5.932±4.711	9.731±0.969	55.261 ±1.449	0.159 ±0.007
R. ide. lty	10.142 ±0.938	1.742±2.610	92.887±3.099	0.239±0.017

* G. pyr. fmt: Flowers of Gentiana pyrenaica L.(Murgul-Tiryal), G. pyr. rmt: roots of Gentiana pyrenaica L.(Murgul-Tiryal), A. dra. lbd: Leaves of Artemisia dracunculus L. (Bayburt-Demirozu), A. dra. sbd: Scapus of Artemisia dracunculus L. (Bayburt-Demirozu), D. kak. fah: Fruits of Diospyros kaki (Artvin-Hatila), D. kak. lah: Leaves of Diospyros kaki (Artvin-Hatila), D. kak. ft: Fruits of Diospyros kaki (Trabzon-Yenikoy), D. kak. ltk: Leaves of Diospyros kaki (Trabzon- Yenikoy), R. ide.fah: Fruits of Rubus ideaus (Artvin-Hatila), R. ide.lah: Leaves of Rubus ideaus (Artvin-Hatila), R. ide.fty Fruits of Rubus ideaus (Trabzon- Yenikoy), R. ide.lty Leaves of Rubus ideaus (Trabzon- Yenikoy).

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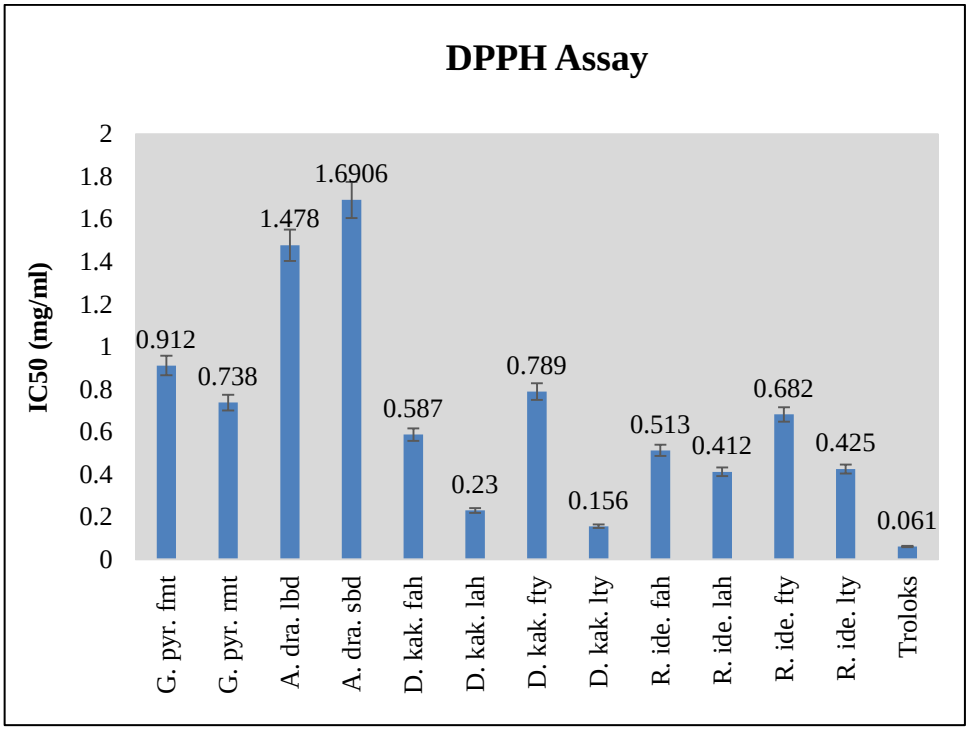

Figure 1: The radical scavenging activity of methanolic extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH●) radical was measured at 517 nm in spectrophometer. The results of DPPH for Gentian Tarragon, Persimmon and Raspberry species.

The antimicrobial activities of extracts assayed against the microorganisms in the present study were qualitatively and quantitatively assessed by evaluating the presence of inhibition zones, zone diameter, and MIC values. The results of antimicrobial activity of methanolic extracts are shown in Table 3.

Table 3. MIC values of compounds against the bacterial strains tested

Samples	Minimal Inhibition Concentration Values (µg/ml)											
	Bs	Ef	Sa	Se	Ec	Kp	Pa	Pv	St	Yp	Ec	Ca
G. pyr. fmt	0,392	-	-	50	-	-	-	-	-	-	-	0.392
G. pyr. rmt	0,196	-	-	50	-	-	-	-	-	-	-	0.392
A. dra. lbd	0,196	-	-	12.5	-	-	-	-	-	-	-	0.392
A. dra. sbd	0.196	-	-	-	-	-	-	-	-	-	--	0.392
D. kak. fah	0,392	-	25	50	-	-	-	50	-	-	-	1.562
D. kak. lah	-	-	-	-	-	-	-	-	-	-	-	-
D. kak. fty	0.196	-	-	100	-	-	-	100	-	-	-	0,196
D. kak. lty	0.196	-	-	25	-	-	-	-	-	-	-	0,196
R. ide. fah	0.392	-	50	100	50	-	-	-	-	-	-	0.780
R. ide. lah	0,780	-	50	100	100	-	-	-	-	-	-	0.780
R. ide. fty	0.196	-	1.25	50	50	-	-	-	-	-	-	0,196
R. ide. lty	0.392	-	25	100	100	-	-		-	-	-	0.392
Kanam.	0.196	6.25	0.782	0.392	1.562	0.392	-	0.196	1.562	0.782	1.562	-

Bs: *Bacillus subtilis* ATCC 6633, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Se: *Staphylococcus epidermidis* ATCC 12228, Ec: *Escherichia coli* ATCC 25922, Kp: *Klebsiella pneumonia* ATCC 13883, Pa: *Pseudomonas aeruginosa* ATCC 27853, Pv: *Proteus vulgaris* ATCC 13315, St: *Salmonella typhimirium* ATCC 14028, Yp: *Yersinia pseudotuberculosis* ATCC 911, Ec: Enterobacter cloaceae ATCC 13047, Ca: Candida albicans ATCC 60193, Kanam.: Kanamycine, (—): no activity of test concentrations

Results obtained from disc diffusion method, followed by measurement of minimum inhibitory concentration (MIC), indicated that B. Subtilis and C. albicans were the most sensitive microorganisms showing lowest MIC values 0.196 µg/ml. Extracts of Gentiana pyrenaica L., Artemisia dracunculus L., Diospyros kaki, Rubus ideaus exhibited antimicrobial activity against the tested strains, but in variable degree. Results are comparable to the antibiotic kanamycine, used as a positive probe.

R. ide. fah, R. ide. lah, R. ide. fty, R. ide. lty and D. kak. fah showed antimicrobial activity against 5 out of 12 microorganisms, D. kak. fty, 4 out of 12 microorganisms, G. pyr. fmt, G. pyr. rmt, A. dra. lbd and D. kak. lty, 3 out of 12 and A. dra. sbd showed antimicrobial activity against 2 out of 12 microorganisms.

Gram positive bacteria were the most sensitive being inhibited by all the extracts except E. faecalis. Concerning Gram negative bacteria, extracts were able to inhibit the growth only E. Coli and P. vulgaris at the extract concentration tested (50-100 µg/ml). Also excellent antimicrobial activity results were observed on the test microorganism, yeast like fungy, Candida albicans (Ca) with the mic values between 0.196 -1.56 µg/ml is better than the standard drug of kanamycine except extract of D. kak. lah.

On the other hand, none of the extracts of plant exhibited the activity on the test microorganisms, E.faecalis, K. pneumonia, P. aeruginosa, S. typhimirium, Y.pseudotuberculosis, E. cloaceae. Extract of D. kak. lah did not shown any antimicrobial activity on the tested microorganisms.

Discussion

Medicinal plants are used in many diseases as reinforcement supplements. In this study, antioxidant and antimicrobial properties of some medicinal plants growing in the black sea region were investigated.

In a study extract on the root of Gentiana asclepiadea L. grown in Serbia, the total phenolic content of extract was 73.51 ± 1.51 mg GAE/g the total flavonoid content was 34.07 ± 0.19 mg QE/g and DPPH activity was 0.24 ± 0.018 mg/ml (28). According to our results extracts on the root of Gentiana pyrenaica L. (belongs to genus Gentiana that comprises about 400 species) grown in Murgul-Tiryal have less total phenolic content, total flavonoid content and DPPH activity.

In a study conducted in India, researches showed that the tested extracts of Gentiane kurroo roots and leaves possessed antibacterial activity against both Gram positive and Gram negative bacteria. The antibacterial activity of root extract was found to be comparatively higher than that of leaf extract. The MIC value of the root extract ranged from 0.15 ± 0.04 to 0.75 ± 0.05 mg/ml and that of leaf from 0.22 ± 0.08 to 0.90 ± 0.02 mg/ml (29). We found that the MIC value of the root extracts of Gentiana asclepiadea L. ranged from 0.196 to 50 µg/ml and that of flowers from 0.196 to 50 µg/ml.

Although several studies have been addressed on some Gentiana species (28, 29, 30) antioxidant and antimicrobial activities of Gentiana pyrenaica L. (roots, leave or flowers) have not been investigated.

In the literature, Artemisia essential oils exhibited weak antioxidant abilities with DDPH radical scavenging activities (31). In the present study, Artemisia dracunculus have showed low DDPH radical scavenging activities according to Trolox standard. While in a study conducted on Artemisia campestris the ability of the extracts to reduce Fe^{3+} was determined 110 ± 2.01 µg/ml according to FRAP assay (32), our result was 18.84 ± 1.16 µmol Fe/g (leave extracts of Artemisia dracunculus L.) and 7.78 ± 026 µmol Fe/g (scapus extracts of Artemisia dracunculus L.). Despite some studies on Artemisia species (31, 32, 33, 34) there has been no research about antioxidant properties of Artemisia dracunculus L. in detailed.

In an article on Artemisia dracunculus, it is reported that whereas Trichophyton rubrum showed the most susceptibility to Artemisia dracunculus extract with growth inhibition zone (20 ± 2.1 mm), Escherichia coli showed the least susceptibility with growth inhibition zone (8 ± 0.0 mm) (31). According to our result, while Artemisia dracunculus extract do not have inhibitory effect on E.coli, it inhibits the growth of S. epidermidis microorganism with MIC value 12.5 µg/ml.

The leaves of Diospyros kaki are commonly used for tea in Asia. Previous studies have shown that persimmon leaves have beneficial effects on the treatment of paralysis, frostbite, and burns, and to stop bleeding (35). It was reported that the extract of Diospyros kaki leaves contained 192 ± 9.6 mg/g total flavonoids and its DPPH radical scavenging activity was 96.36 ± 2.63 µg/ml (36). We found that total flavonoids of the extract of Diospyros kaki leaves is 84.2±2.5 mg/g and its DPPH radical scavenging activity is 230 µg/ml . In addition to that, D. The flavonoid contents of the other samples named as kak. fah, D. kak. fty and D. kak. lty are 9.731±0.969, 0.457±0.053 and 64.512±4.153 mg/g and their DPPH radical scavenging activities are 587, 789 and 156 µg/ml, respectively.

In an article on Diospyros kaki, the extract of persimmon peel did not exhihit potent anti-Helicobacter pylori activity (MIC>100 µg/ml) (37). In this research, it is found that various samples of Diospyros kaki exhibit inhibitory effect on Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris with MIC values ranging from 0.196 to 100 µg/ml.

In the literature extract on the pomaces of Rubus idaeus, researches determined that the total phenolic content of extract was 26.3 ± 1.28 mg GAE/g the total flavonoid content was 25.2 ± 1.20 mg QE/g and DPPH activity was 3.86 ± 0.18 mg/ml (38). In our study extract on the fruits of Rubus idaeus grown in Artvin, the total phenolic content of extract was 6.05 ± 0.61 mg GAE/g the total flavonoid content was 10.98 ± 0.27 mg QE/g and DPPH activity was 0.51 mg/ml. In addition extract on the fruits of Rubus idaeus grown in Trabzon, the total phenolic content of extract was 5.93 ± 4.71 mg GAE/g the total flavonoid content was 9.73 ± 0.97 mg QE/g and DPPH activity was

0.68 mg/ml. There are several studies in the literature about antioxidant activity on fruits of Rubus idaeus species (38, 39, 40) however there are a few studies on the leaves of Rubus idaeus. Therefore, antioxidant activities of the leaves of Rubus idaeus was also investigated in this study.

In a research pomace extract of Rubus idaeus showed significantly higher activity towards reference E.coli and wild L.monocytogenes showing MIC values 0.29 mg/ml and 0.39 mg/ml, respectively (38). According to our study extract of Rubus idaeus both fruits and leaves exhibited higher activity towards effect on B. subtilis and C. albicans with MIC values ranging from 0.196 to 0.78 µg/ml.

The observed differences between our study and the study of Dragana et al. (38) are certainly caused by the activity is due to the composition and amount of active components and is dependent on genetic (i.e. genus, species, cultivar/genotype) and environmental factors, such as geographic areas, growth conditions of plant material, seasonal variations, climatic factors, ripening stage, harvesting time, storage condition and postharvest management (41, 42, 43). Solar radiation, temperature, virus status, and other biotic and abiotic stresses also affect phenolic content (43).

Based on these results, it is possible to conclude that methanolic extracts of Gentiana pyrenaica L., Artemisia dracunculus L., Diospyros kaki, Rubus ideaus had different levels of antioxidant and antimicrobial activity. The obtained results might be considered sufficient to further studies for the isolation and identification of the active principles and to evaluate of possible synergism among extract components for their antioxidant and antimicrobial activity. Investigations are in progress to determine the degree of toxicity of these extracts.

Conclusion

The findings of this study indicate that the plant extracts of Gentian, Tarragon, Persimmon and Raspberry contain compounds with antioxidant activity, antimicrobial and antifungal activity. The replacement of synthetic with natural antioxidants may be advantageous. Based on these results, it is possible to conclude that methanolic extracts of these plants can be the potent source of natural antioxidants.

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Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: SC, BH, OS, MO, ES: Protocol or project development, Data collection, Biochemical Analysis**. SC:** Data analysis Manuscript editing or writing, Revisions. All authors approved the final version of the manuscript.

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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Effects of melatonin and agomelatine on doxorubicin induced anxiety and depression-like behaviors in rats

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Abstract

Objective: Doxorubicin (DOX) is a chemotherapeutic agent used to treat several cancer types; however, it exhibits severe side effects in the nervous system which DOX treatment evoked neurobehavioral alterations such as anxiety and depressive-like behavior. We investigated the use of melatonin and agomelatine to prevent neurobehavioral alterations caused by DOX.

Material and Methods: Forty-nine Wistar albino rats were randomly divided into 7 groups, namely control (CON, n=7), doxorubicin (DOX, n=7), melatonin (MEL, n=7), agomelatine (AGO, n=7), melatonin + doxorubicin (MEL + DOX, n=7), agomelatine + doxorubicin (AGO + DOX, n=7) melatonin + agomelatine + doxorubicin (MEL + AGO + DOX, n=7) groups. Doxorubicin (18 mg/kg) was injected intraperitoneally (i.p) on the 5th, 6th, 7th day of the study. Animals were treated with melatonin (40 mg/kg/i.p), agomelatine (40 mg/kg/i.p), melatonin (40 mg/kg/i.p) + agomelatine (40 mg/kg/i.p), for 7 days and then doxorubicin (18 mg/kg/i.p) was injected on the 5th, 6th, 7th day. On the 8th day of the experiment, all animal evaluated open field test (OFT) and forced swim test (FST) respectively.

Results: The only DOX-treated rats exhibited the reduced exploration, grooming, and locomotor activity in the open field test and increased immobility time, reduced swimming time. Our data showed that the rats treated with DOX exhibited anxiety and depressive-like behavior. Melatonin and agomelatine treatment reduced all the parameters of DOX-induced anxiety and depressive-like behavior in rats.

Conclusions: Melatonin and agomelatine have a protective effect of against DOX-induced neurobehavioral alterations in rats.

Keywords: Doxorubicin, Melatonin, Agomelatine, Rat, Anxiety, Depression

Introduction

Cancer incidence is increasing with advancing age. It is estimated that nearly 70% of individuals over the age of 65 can get cancer by 2030 (1). Due to its persistent condition, cancer disease represents a major global health problem (2). A variety of therapeutic approaches, including antineoplastic drugs, chemotherapy, and antihormonal therapies have been used to treat cancer. Doxorubicin (DOX) is a powerful antineoplastic agent (3). The use of DOX for the treatment of various types of cancer is supported by experimental and clinical data (4,5). Although they are seen as a promising target for the development of medications, clinical and preclinical studies have shown that DOX and other antineoplastic drugs generally produce an undesirable effect in the cardiovascular system and central nervous system (6,7,8,9).

Recent animal studies revealed DOX-evoked neurobehavioral alterations such as anxiety and depression, limiting the optimization of doses in clinical trials and preclinical studies (10,11).

Agomelatine is a melatonergic M1 and M2 receptor agonist and serotonergic (5-HT2C) receptor antagonist properties (12). Agomelatine also shows a longer half-life and greater affinity for MT1 and MT2 melatonin receptors (13). Melatonin is a powerful antioxidant. In recent studies, Melatonin showed the anxiolytic effect and neuroprotective property in the experimental animal model (14,15,16).

The effect of melatonin and agomelatine against DOX-induced neurobehavioral changes has not been studied so far.

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Therefore, in the present study, we have investigated the possible neuroprotective effects of agomelatine alone and in combination with melatonin against DOX-induced neurotoxicity. We have determined the neurobehavioral changes (anxiety and depression), using Open Field Test (OFT) and Forced Swimming Test (FST).

Materials and Methods

Chemicals

Doxorubicin hydrochloride injection was purchased from Sandoz Pharmaceutical Industry, Turkey. Melatonin and agomelatine were commercially obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA).

Animals

Male Wistar rats (N=49; n=7 per group, aged 8-10 weeks; weighing 200–250 g, from the animal facility of the Gaziosmanpaşa University Animal Experimental Center, Tokat, Turkey, were used. The animals were housed under standard conditions of temperature (23°C±2°C) light, relative humidity (65 ± 10 %) and (12:12 h light/dark cycle) and with free access to water and food. All animals were maintained in individually ventilated Hepa filter cages. Daily health checks were carried out by the veterinarian. The animals were housed –cages for 7 days (pre-experimental period) to habituate prior to all drug injection and behavioral testing. All experimental procedures took place in the same room in which the habituation took place. Testing equipment had been installed in this room prior to the arrival of the animals. The animal room was sound-attenuated. All animal use procedures were carried out in accordance with the Regulations of Experimental Animal Administration. At the end of the experiment, all rats were killed by cervical dislocation under anesthesia. The study was approved and carried out under the strict rules structured by Institutional Animal Ethics Committee.

Experimental Design

The animals were randomly divided into seven groups of seven rats each (n=7x7):

Group I (Control) served as control groups and animals received saline for 7 days.

Group II (DOX) served as DOX groups, in which the animals received a total cumulative dose of 18 mg/kg, body weight, i.p. of DOX for 5th, 6th and 7th days.

Group III (MEL) animals received melatonin (40 mg/kg body weight, i.p.) for 7 days, dissolved in saline.

Group IV (AGO) animals received agomelatine (40 mg/kg body weight, i.p.) for 7 days, dissolved in saline.

Group V (MEL + DOX) animals received melatonin (40 mg/kg body weight, i.p.; dissolved in saline) for 7 days and were injected with DOX (cumulative dose: 18 mg/kg, i.p.) on the 5th, 6th and 7th days.

Group VI (AGO + DOX) animals received agomelatine (40 mg/kg body weight, i.p.; dissolved in saline) for 7 days and were injected with DOX (cumulative dose: 18 mg/kg, i.p.) on the 5th, 6th and 7th days.

Group VII (MEL + AGO + DOX) animals received melatonin and agomelatine (40 mg/kg body weight, i.p.; dissolved in saline) for 7 days and were injected with DOX (cumulative dose: 18 mg/kg, i.p.) on the 5th, 6th and 7th days.

Behavioral Assessment

Open Field Test

On the eighth day of the experiment, all groups were subjected to open field testing. The spontaneous locomotor activity of rats was tested on an area of 100x100 cm divided into 64 equal cuts in the open area. The movements of the animals were recorded with a video camera. Behavioral characteristics of the animals were assessed for 5 minutes on the open field. During this time, the following behavioral parameters were measured: locomotor activity (the number of squares crossed), the number of rearings and the duration of groomings. After each test, the animals were returned to their home cages, and the apparatus was cleaned with an alcoholic solution (5% alcoholic) followed by wet and dry paper towels (17).

Forced Swim Test

On the eighth day, rats were individually placed into Plexiglas cylinders (54 cm high; length, 34 cm; width 60 cm) filled with water (24.0±1 °C) to a depth of 40 cm. Test sessions were recorded by a video camera positioned directly above the cylinders (18). Rats were forced to swim for 5 min. During this time, the following behavioral parameters were measured: the time spent in immobility and swimming.

Statistical Analysis

Results are presented as the mean±standard error of the mean (SEM). The data were analyzed with One-way analysis of variance (ANOVA) used to compare key variable between groups, followed by the posthoc Tukey. Statistical significance was considered with a $p<0.05$. All statistical analyses were processed with Graph Pad Prism 7.0 software.

Results:

Open Field Test

DOX-induced anxiety-like behavior in rats was evaluated through OFT.

The DOX-treated groups and the melatonin, agomelatine, melatonin combination with agomelatine pre-co-treatment in the DOX-treated group, showed significantly decreased the number of squares crossed (Figure 1A, $p<0.001$), the number of rearings (Figure 1B, $p<0.001$), and the duration of grooming (Figure 1C, $p<0.001$) when compared to control groups (Table 1).

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The melatonin pre-co-treatment in the DOX-treated group show significantly enhanced the number of squares crossed (Figure 1A, p<0.001), the number of rearings (Figure 1B, p<0.001 and p<0.05 respectively) and the duration of grooming (Figure 1C, p<0.001) when compared to the DOX-treated group.

The agomelatine pre-co-treatment in the DOX-treated group show significantly enhanced the number of squares crossed (Figure 1A, p<0.001), the number of rearings (Figure 1B, p<0.001) and the duration of grooming (Figure 1C, p<0.001) when compared to the DOX-treated group (Table 1).

The melatonin and agomelatine combination pre-co-treatment in the DOX-treated group show significantly enhanced the number of squares crossed (Figure 1A, p<0.001), the number of rearings (Figure 1B p<0.01) and the duration of grooming (Figure 1C, p<0.001) when compared to the DOX-treated group (Table 1).

Forced Swim Test

DOX-induced depressive-like behavior in rats was evaluated through Forced FST.

The DOX-treated groups showed the significantly increased immobility time (Figure 2A, p<0.001) and decreased the swimming time (Figure 2B, p<0.001) when compared with the control group (Table 2).

The melatonin, agomelatine and melatonin combination with agomelatine pre-co-treatment in the DOX-treated group, did not show any significant changes in immobility time and swimming time when compared with the control group (Figure 2A, 2B, Table 2).

The melatonin, agomelatine and melatonin combination with agomelatine pre-co-treatment in the DOX-treated group, show significantly decreased the immobility time (Figure 2A, p<0.001; p<0.001; p<0.001 respectively) and increased the swimming time in FST (Figure 2B, p<0.01; p<0.001, p<0.001 respectively) when compared to DOX-treated groups (Table 2).

Table 1. OFT parameters show that CON (control), DOX (doxorubicin), MEL (melatonin), AGO (agomelatine). Data are presented as mean±SEM. One-way ANOVA with post-hoc Tukey test was used. (a=p<0.05, b=p<0.01, c=p<0.001) compared to the control group; (d=p< 0.05, e=p<0.01, f=p<0.001) compared to the DOX alone-treated group.

Groups	Number of squares crossed	Number of rearings	Duration of grooming
CON	85.17±2.94	21±0.89	29.5±1.52
DOX	26.5±3.64;c	5.33±0.84;c	8.66±1.02;c
MEL	90.83±2.78	22.17±1.01	30.33±1.17
AGO	89.83±4.07	23.5±0.95	31.83±1.24
MEL+DOX	57.5±3.61;c,f	8.66±0.95;c,d	18.67±0.88;c,f
AGO+DOX	6.5±3.75;c,f	10.17±1.07;c,e	20.17±0.54;c,f
MEL+AGO+DOX	62.83±3.47c,f	11.33±0.95;c,e	22.83±1.16;c,f

Table 2. FST parameters show that CON (control), DOX (doxorubicin), MEL (melatonin), AGO (agomelatine). Data are presented as mean±SEM. One-way ANOVA with post-hoc Tukey test was used. (a= p< 0.05, b=p<0.01, c=p<0.001) compared to the control group; (d= p< 0.05, e=p<0.01, f=p<0.001) compared to the DOX alone-treated group.

Groups	Immobility Time (s)	Swimming Time (s)
CON	147.3±2.57	71.5±1.97
DOX	169±1.71;c	54.5±0.88;c
MEL	139.3±3.59	75.67±3.01
AGO	136±5.27	78±3.17
MEL+DOX	142.5±2.01;f	65.17±2.28;b,e
AGO+DOX	150±1.09;f	69.33±1.78;c,f
MEL+AGO+DOX	151±1.67;f	70±1.82;c,f

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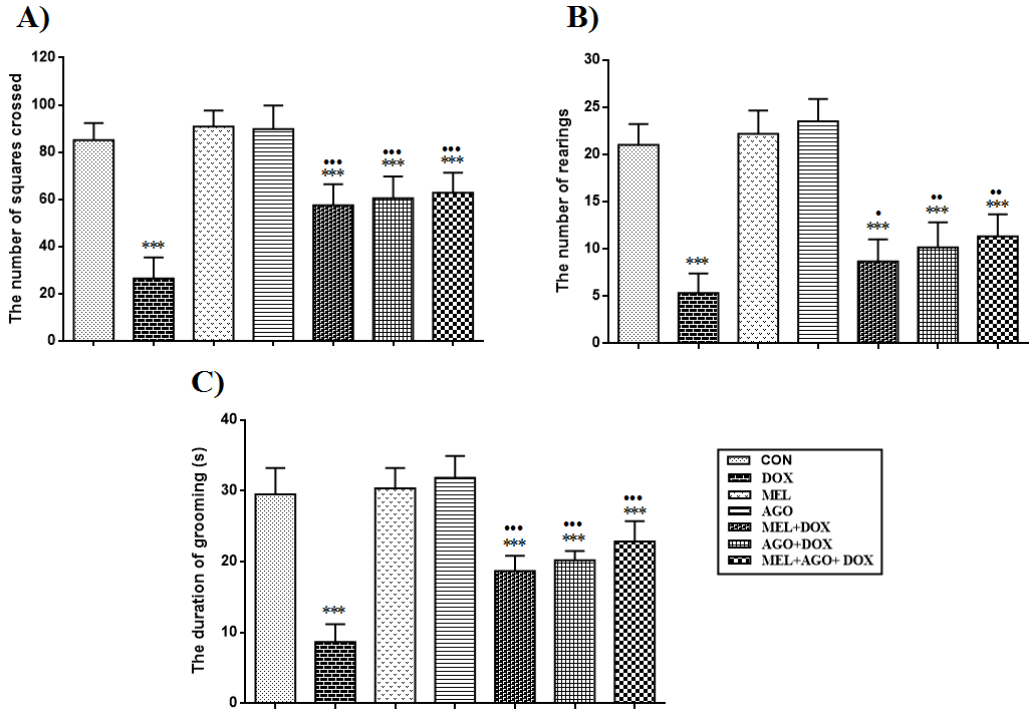

Figure 1. Melatonin and agomelatine effect on OFT parameters in DOX-pretreated rats. A) locomotor activity (the number of squares crossed) B) the number of rearings, C) the duration of groomings. Data are as mean± SEM. One-way ANOVA and Tukey test. (*=p<0.05,**=p<0.01,***=p<0.001) compared to the control group; (• =p<0.05, (•• =p<0.01), (••• =p<0.001), CON (control) compared to the DOX alone-treated group; DOX (doxorubicin), MEL (melatonin), AGO (agomelatine).

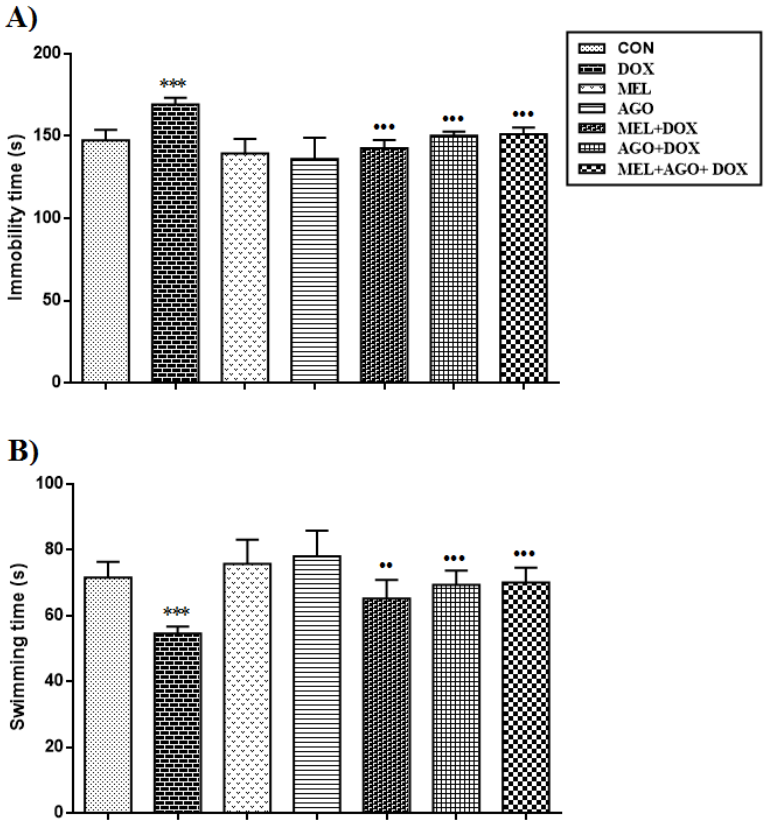

Figure 2. Melatonin and agomelatine effect on FST in DOX-pretreated rats. A) Immobility time (P < 0.05), B) swimming time. Data are as mean± SEM. One-way ANOVA and Tukey test. (*=p<0.05,**=p<0.01,***=p<0.001) compared to the control group; (• =p<0.05, (•• =p<0.01), (••• =p<0.001), CON (control) compared to the DOX alone-treated group; DOX (doxorubicin), MEL (melatonin), AGO (agomelatine).

Discussion

In the present study, it is showed that DOX injection induced the behavioral changes in anxiety and depression. Studies have shown that the affinity of agomelatine for melatonin receptor is higher than for serotonin 5-HT2C receptors. For this reason, the study was intended to compare melatonin in equal doses. Thus, we observed that whether or not there was a behavioral difference in rats when agomelatine and melatonin were administered at the same dose. Subchronic and systemic administration of agomelatine doses showed a considerable anxiolytic and antidepressive effect similar to melatonin on DOX-induced behavioral changes in rats.

OFT has been widely used to assess anxiety-like behavior in rodents. In our studies, locomotor activity (in the number of squares crossed), exploratory behavior (the number of rearings) and grooming reactions in the DOX-treated rat were lower than control rats in OFT. The decrease in locomotor activity means an increase in anxiety-like behavior. (19,20). Decreased exploratory behavior may represent an implying deficit in novelty seeking motivation, loss of interest in new situations, a symptom of anxiety and depressive disorder. Decreased number of grooming reactions seem to submissive social behavior, social neglect and maternal cannibalism, mimic a loss of interest in or pleasure from typically, a core symptom of depression (21, 22, 23, 24). The FST was chosen to test a behavioral measure of depressive-like state. Immobility time has been characterized as behavioral despair (25). In this study, treatment with the doxorubicin significantly increased immobility time and decreased the swimming time compared to control rats. According to our findings, DOX-treated rats are more anxious and depressive than control rats.

Long-term use of DOX triggers neurotoxicity and may cause neuropsychiatric diseases including anxiety and depression (26). In the OFT, DOX-treated rat showed a reduced exploratory behavior and locomotor activity. Also, it leads to the behavior changes in rats (27,28). Moreover, a single injection low dose of DOX (7 mg/kg) enhanced immobility time and decreased swimming time. Thus DOX administration could be associated with a mild depressive-like behavior.

The molecular mechanisms underlying the anxiety - depressive-like behavior in DOX-injected rats were associated with the increased brain oxidative stress and reduced total antioxidant capacity (10, 29, 30, 31, 32, 33).

Melatonin is the mainly endogenous antioxidant. It has a function to reduce the oxidative stress levels of a cell and to try to scavenge free radicals to prevent cell damage and neuronal death (34). Montilla et al. (35) found that DOX injection increased the oxidative stress, which was reduced by melatonin, in the hypothalamus and brain cortex. The antidepressant and anxiolytic effects of melatonin have been previously described in rodents subjected to the FST and OFT (36,37). In the present study, we demonstrated that an increase depressive-like behavior (significant increase immobility time and decreased swimming time) and anxiety-like behavior (significant decrease locomotor activity, exploratory behavior, and grooming reactions) in following DOX administration was significantly prevented by melatonin.

Agomelatine is a new antidepressant drug, an agonist at MT1, MT2 receptors, and antagonist at 5-HT2C receptors was the first melatonin receptor ligand showing antidepressant-like activity in animal drug screening tests (38). The affinity of agomelatine for the 5-HT2C receptor is in the micromolar range and about 100-fold less than its affinity for melatonin receptors (13). Melatonin receptors are involved in mediating anhedonic- and anxiety-like behaviors (39). Our findings demonstrate that agomelatine attenuates the DOX-related anxiety and depressive-like behavior. The repressive effect of agomelatine on the DOX-induced behavioral changes in rats was found to be similar to that of melatonin. The results of the present study point out that the antidepressant effect of agomelatine may have been by antioxidant activity. As a result, agomelatine treatment may be helpful in managing depression and showed strong efficacy in the various animal depression model (40,41). Also, it should be considered that in many studies, agomelatine might modulate depression-induced lipid peroxidation and pro-inflammatory cytokines in the brain, kidney, and liver (42,43,44). However, agomelatine stimulates cytokine production in the kidney (44).

Conclusion

The present study demonstrates that melatonin and agomelatine treatment was able to reduce DOX-induced anxiety and depressive-like behavior evaluated in the OFT and FST. Thus, the antidepressant drugs must provide therapeutic potential without the risk of adverse effects, making it a valuable tool for the treatment of depression related to the use of antineoplastic drugs.

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Ethical issues: All Authors declare originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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Neuroprotective effects of boric acid against fluoride toxicity on rat synaptosomes

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Abstract

Objective: Fluoride toxicity primarily contributes to the production of reactive oxygen and nitrogen derivatives, trigger the cell death pathways by causing lipid peroxidation and DNA damage. Boric acid (BA) contributes to preservation of membrane integrity and function and maintenance of redox balance due to its high affinity to some metabolites in the organism. The aim of this study was to investigate the protective effect of BA on neurodegenerative processes against the toxic effects of sodium fluoride (NaF) administered at different doses on rat brain synaptosomes.

Material and Methods: Synaptosomes obtained from the rat frontal cortex were administered at different doses of sodium fluoride (NaF) to determine the most toxic dose of NaF. Determined toxic dose of NaF for synaptosomes and BA concentrations were administered in vitro at 37°C for 30min and then the parameters of malondialdehyde (MDA) level, superoxide dismutase (SOD) activity, Na/K ATPase activity and DNA fragmentation value were measured spectrophotometrically.

Results: There was a statistically significant difference between measured parameters, when the 80mg/L NaF group was compared with the control group. We found that 10 and 25 mM BA treatment provided a significant improvement in MDA, SOD, Na/K ATPase and DNA fragmentation compared to the 80mg/L NaF group. The 5 mM BA concentration was not found effective dose according to other doses.

Conclusion: In conclusion, BA has potential for neuroprotective effects against cellular damage caused by NaF. The results suggest that the BA can be a neuroprotective therapeutic agent for fluoride toxicity.

Key words: Sodium fluoride, Synaptosomes, Boric acid, Neuroprotection.

Introduction

Fluoride (F), chemically ionic element, can produce free oxygen and nitrogen radicals (ROS and RNS, respectively) by affecting the antioxidant metabolism (1). Due to the electronegative structure of F, which means that it is negatively charged and tends to form fluorine ions, it can pass through cell membranes via ion channels (2). Excessive F uptake causes fluorosis, an important health problem, which is characterized by defects in skeletal and tooth structure (3). The main cause of fluorosis is contaminated drinking water with organic and inorganic wastes. Since F in drinking water has an ionic structure, it is absorbed rapidly through the intestinal epithelium and interferes with metabolic processes by accumulating in the different organs of the biological systems (4). In vivo studies have found that F added to drinking water of rats causes toxic effects and accumulates in soft tissues such as lung, liver, heart, brain and kidney (5).

Furthermore, F-induced ROS production reduces glutathione (GSH) levels as well as inhibition of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (6). Increasing lipid peroxidation is also an important biomarker of oxidative stress. In vivo studies have been shown that F exposure enhances lipid peroxidation due to increased ROS production in rat brain tissues (7). Besides, F exposure has been shown to cause genotoxic effects with chromosome anomalies and DNA damage (8).

Synaptosomes as a prototype of nerve tissue can fulfill many different metabolic functions. They are widely used owing to high mitochondrial contents, easy preparation and demonstration of synaptic functions (9). Synaptosomes have provided valuable information about the molecular mechanisms underlying neurotransmitter release, aging,

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and the pathogenesis of neurodegenerative diseases, and thus become a useful tool for monitoring molecular and bioenergetic changes in synapses (10). Synaptosomes are highly vulnerable to oxidative stress due to high unsaturated fatty acid content and high oxygen consumption such as brain (11).

Boric acid (BA) is a compound found in living organisms as a trace element. The boric acid, which is a common form of boron, is soluble in water and can incorporate into biochemical processes. BA, a monobasic molecule, contains hydroxyl groups in the chemical structure and releases protons during the reaction (12). Owing to high affinity of BA to some important molecules involved in biochemical and physiological processes such as nicotinamide adenine dinucleotide, flavin adenine dinucleotide, glycolipids, glycoproteins, and oxidoreductases may play an important role in cell membrane integrity and redox metabolism. BA is used in a lot of fields from industry to agriculture. When products containing BA are consumed, it rapidly crosses the bloodstream through the gastrointestinal tract (13). Recent studies have provided evidence that BA can be used in the treatment of some types of cancer (14). Previous studies have reported that BA has protective effects against inflammatory and oxidative damage (15). BA is involved in hormone metabolism, transmembrane signaling, and various enzymatic systems and acts as an antioxidant (16,17).

In this study, we aimed to investigate the neuroprotective effects of BA against F toxicity on account of the increase in the prevalence of studies on fluorosis in recent years. To verify our hypothesis, malondialdehyde (MDA) levels, SOD activities, DNA fragmentation and Na/K ATPase activities were measured to reveal the neuroprotective effects of BA following sodium fluoride (NaF) exposure of rat brain synaptosomes.

Material and Methods

Animals and Experimental Design

Eight healthy male Wistar albino rats (weighing 250±50 g) were supplied by Medical and Surgical Experimental Animal Applications and Research Center, Eskisehir. Experimental procedures were carried out according to the decision of Experimental Animals Ethics Committee of Eskisehir Osmangazi University (Approval number: 650). The rats were maintained under controlled conditions at 22°C ± 5°C and 45% ± 5% relative humidity with 12-hour periods (dark / light). Anesthesia was performed by intra-muscular injection at 45±5 mg/kg ketamine + 10±2 mg/kg xylazine doses, and then the unconscious rats were decapitated. Rats' frontal cortex was removed and divided into 4 equal cuts and the cuts were stored at -80°C until the day of the experiment.

In this study we investigated the neuroprotective effects of BA at 5, 10 and 25 mM concentrations versus the toxicity caused by NaF. We first researched which of the 20, 40 and 80 mg/L NaF doses were more toxic. We then administered different doses of BA treatments after determining the most toxic dose of NaF on the synaptosomes.

Preparation of synaptosomal fractions

In this study, crude synaptosomal fractions were prepared according to the modified method of Whittaker et al (18). Brain cuts from previously healthy rats were distributed randomly to experimental groups as 6 cuts in each group (n=6). The cuts were homogenized on ice with 10 mM 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) and 30 µM sucrose. The homogenates were first centrifuged at 3000xg for 10 minutes at 4°C and then the supernatants were taken and centrifuged once more at 15000xg for 20 min at 4°C. The remaining pellets were re-suspended in saline and rat brain synaptosomal fractions were obtained. According to the determined experimental groups, synaptosomes were exposed to at 20, 40 and 80 mg/L NaF and 5, 10 and 25 mM BA (Sigma, B6768, Germany) concentrations for 30 minutes at 37°C. Synaptosomal protein levels were measured according to the biuret method (19).

This method is used to demonstrate the presence of peptide bonds in the samples. The reaction of Cu+2 with the two peptide bonds is based on the principle of purple color formation, and the reaction product chelate product is measured spectrophotometrically at 540 nm.

Measurement of synaptosomal malondialdehyde (MDA) levels

The quantitative determination of lipid peroxidation is based on the color reaction between MDA and thiobarbituric acid (TBA). Synaptosomal MDA levels were measured at 532 nm according to the method reported by Ohkawa et al (20). In short, 0.6 ml rat synaptosomal fraction was added to sample 4 ml of sodium dodecyl sulphate (8%; Merck, 817034 Germany), and then 2 ml of acetic acid (% 0.6, pH 6.5; Merck, 100063, Germany) and 2 ml thiobarbituric acid solution (% 20, pH 4; Merck, 108180, Germany) was added to the reaction medium. The final concentration was adjusted to be 5 ml and heated in a water bath at 100°C for 60 minutes. After this process, it was centrifuged at 4000 rpm for 10 minutes and then spectrophotometric measurement was performed. The results were expressed as nmol/mg protein.

Measurement of synaptosomal superoxide dismutase (SOD) activities

SOD activity in liver tissue was measured according to the method of Sun et al (21). Briefly, the determination of SOD activity is based on the inhibition of nitro blue tetrazolium (NBT, Sigma, 74032, Germany) reduction of super oxide anion resulting from reaction of xanthine with xanthine oxidase. The reaction was started by adding 50 µl of xanthine oxidase to the reaction medium. Superoxide dismutase activity was measured spectrophotometrically at 560 nm for 5 min. One unit SOD was defined as the enzyme amount causing 50% inhibition of NBT reduction. The result were indicated as Unit/mg protein.

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Measurement of synaptosomal Na/K ATPase activities

Na/K ATPase activities were initiated by adding 5 µl of synaptosomal fraction to the reaction medium. Subsequently, nicotinamide adenine dinucleotide (Sigma, N1636, Germany) oxidation was measured at 340 nm for 10 minutes at intervals of 30 seconds (22). The data were indicated as U/mg protein.

DNA fragmentation values

DNA fragmentation was performed spectrophotometrically at 660 nm by reaction of the synaptosomal fractions with diphenylamine, and the data were indicated as a ratio of pellet to supernatant (23).

Statistical analyzes

Data obtained from experimental studies were evaluated using SPSS 21.0 Windows program. One-way ANOVA test was used to determine whether the results were statistically significant (P <0.05). Post hoc Tukey HSD test was used for comparison among the experimental groups.

Results

As shown in Figure 1, NaF exposure caused an increase in lipid peroxidation on synaptosomes, thus increasing MDA levels. MDA levels of 80 mg/L NaF group were significantly higher than control group (P <0.01). 5 mM and 10 mM BA concentrations treatment provided an amelioration by reducing effect at MDA levels, while 25 mM BA concentration treatment group was almost obtained similar results to the control group (P <0.001). In addition, we can say that the increase in MDA levels against fluoride toxicity showed a dose-dependent decrease with BA treatment.

NaF exposure was found to cause a significant decrease in SOD and Na/K ATPase activities on synaptosomes compared to the control group (Figure 2 and 3). BA treatment provided protective effect against NaF toxicity and increased SOD and Na/K ATPase activities. 25 mM BA concentration among all doses resulted in the most improvement in SOD and Na/K ATPase activities against 80 mg/L NaF toxicity group (P <0.001). However, 5 mM BA concentration did not provide a significant difference in SOD and Na/K ATPase activities compared with the 80 mg/L NaF group (P >0.05).

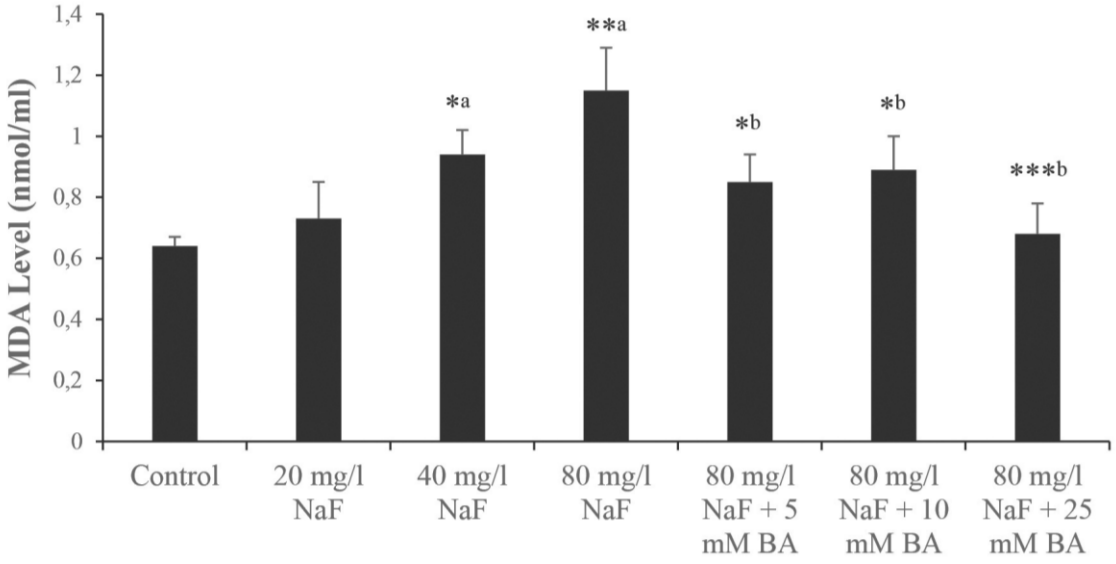

Figure 1: The neuroprotective effects of BA on MDA levels against NaF-induced toxicity on rat brain synaptosomes. All data are expressed as mean ± SEM (n=6 in each group). a: As compared to control group. b: As compared to 80 mg/L NaF group. *P <0.05, **P <0.01, ***P <0.001. NaF: Sodium fluoride. MDA: Malondialdehyde. BA: Boric Acid.

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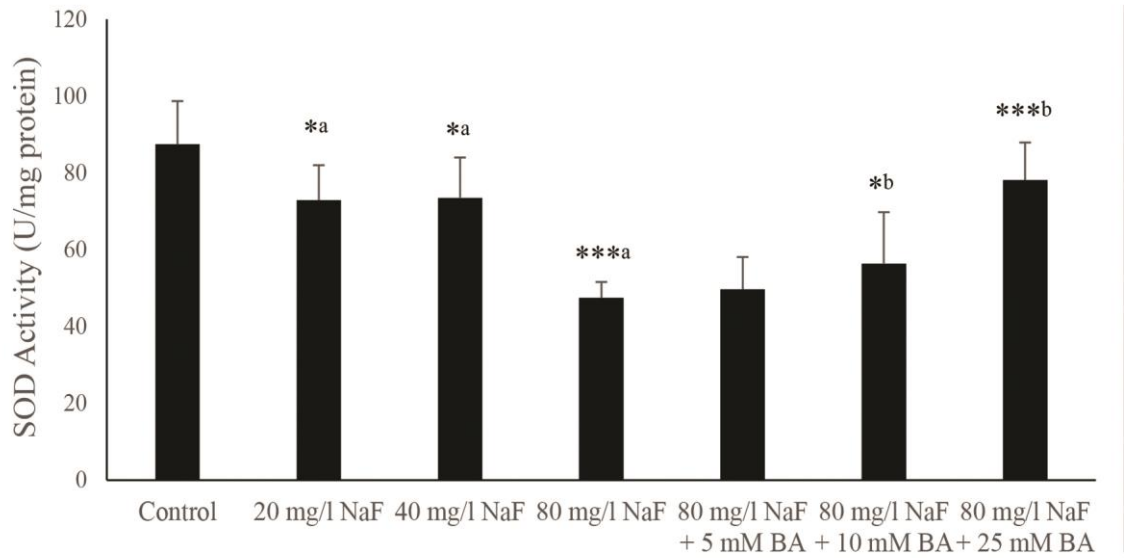

Figure 2: The neuroprotective effects of BA on SOD activity against NaF-induced toxicity on rat brain synaptosomes. All data are expressed as mean ± SEM (n=6 in each group). a: As compared to control group. b: As compared to 80 mg/L NaF group. * P <0.05, *** P <0.001. NaF: Sodium fluoride. SOD: Superoxid dismutase. BA: Boric Acid.

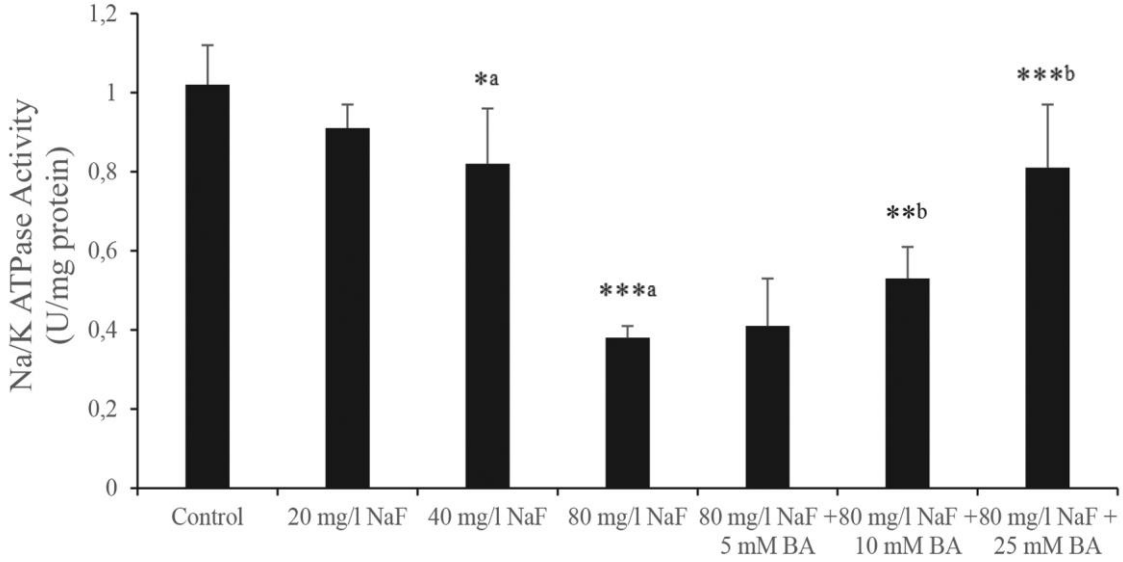

Figure 3: The neuroprotective effects of BA on Na/K activity against NaF-induced toxicity on rat brain synaptosomes. All data are expressed as mean ± SEM (n=6 in each group). a: As compared to control group. b: As compared to 80 mg/L NaF group. * P <0.05, ** P <0.01, *** P <0.001. NaF: Sodium fluoride. BA: Boric Acid.

The 10 and 25 mM BA concentrations treatment resulted in a statistically significant reduction in DNA fragmentation value compared with the 80 mg/L NaF group (P <0.01). On the other hand, 80 mg/L NaF+5 mM BA group did not cause a statistically significant decrease in DNA fragmentation (P>0.05).

As shown in Figure 4, the highest decrease/improvement in DNA fragmentation levels after NaF exposure was obtained at 25 mM BA concentration (P <0.001).

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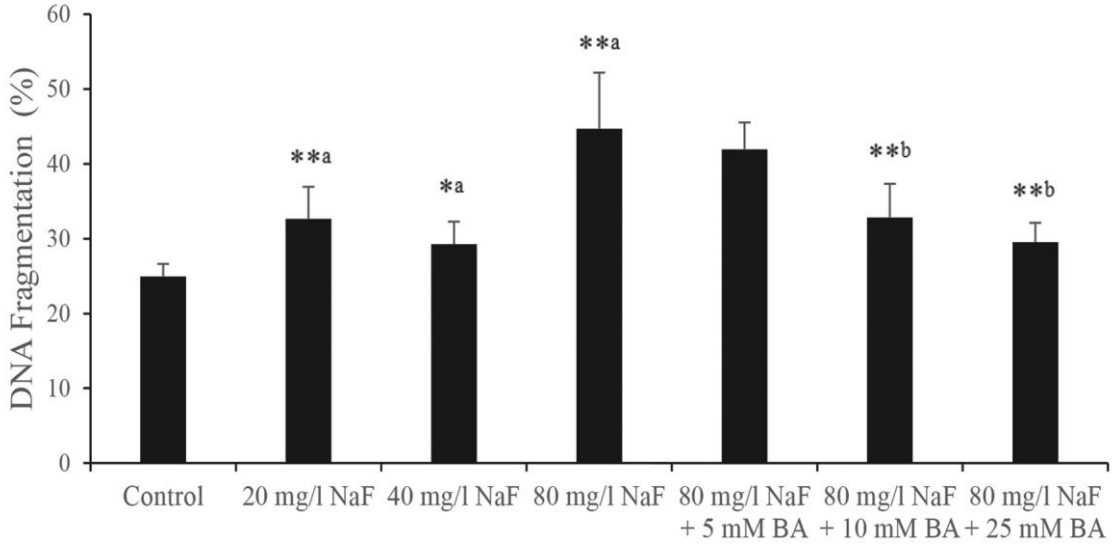

Figure 4: The neuroprotective effects of BA on DNA fragmentation against NaF-induced toxicity on rat brain synaptosomes. All data are expressed as mean ± SEM (n=6 in each group). a: As compared to control group. b: As compared to 80 mg/L NaF group. * P <0.05, ** P <0.01. NaF: Sodium fluoride. BA: Boric Acid.

Discussion

In this study, neurotoxic effect of F on rat brain synaptosomal fractions and also the evolution of neuroprotective effects of BA were investigated. Especially, 80 mg/L NaF exposure were found to cause cellular damage by triggering oxidative stress. It has been determined that the antioxidant capacity also reduce due to increased reactive oxygen species by F exposure in synaptosomes. Since the F chemical structure is highly electronegative, in vitro and in vivo studies have been shown to cause oxidative stress-induced cellular damage by up-regulation of reactive oxygen species (24,25).

Oxidative stress is associated with neurotoxicity of unsaturated long chain fatty acids occurring in mitochondrial dysfunction and neurodegenerative diseases (26). Synapses that play an important role in neuronal signal transduction are highly correlated with oxidative stress-induced neurotoxicity. Synaptosomes are highly vulnerable to lipid and protein oxidation due to their high mitochondrial content and energy consumption in presynaptic neuronal axons.

Therefore, we examined the neuroprotective effects of BA, an important antioxidant, against NaF toxicity. Our results consistent with the literature, we found that NaF exposure increased MDA levels in synaptosomes by increasing oxidative stress (27,28).

Also we found that NaF-induced increased MDA levels showed a reduction with BA treatment. This suggests that BA acts as a potential antioxidant against lipid peroxidation.

SOD, a component of the antioxidant mechanism, plays an essential role in protecting cellular integrity against peroxidative damage resulting from ROS (29). İn vivo studies have been showed that BA contributed to antioxidant mechanism by providing upregulation of SOD against increased oxidative stress (30). BA treatment on rat brain synaptosomes has significantly improved antioxidant enzyme levels due to its free radical scavenger effects. In other words, BA helps protect cellular integrity by supporting antioxidant defense system. Similar to our results, decreased SOD activity due to increased oxidative stress showed an increase with BA treatment (31).

Na/K ATPase is a membrane protein that plays an important role in maintaining the electrochemical membrane potential in cells. It is also involved in the provision of intracellular and extracellular electrolyte balances (32). F binds to the proteins of ion channels in cell membranes and inhibits them, causing the deterioration of membrane potential (33). F exposure has been reported to inhibit Na/K ATPase activity in brain tissue (34). In our study, Na/K ATPase activities in synaptosomal fractions were reduced after NaF exposure, and then BA treatment was improved in Na/K ATPase activities.

F exposure has been found to cause chromosomal abnormalities by increasing the frequency of micronucleus and gene mutations in cell lines (35). Previous studies have reported that oxidative stress, DNA damage, activation of apoptotic pathways and cell cycle changes were induced by fluoride in rats (36,37).

Zhang et al. (38) reported that 80 mg/l fluoride exposure in rat hippocampal neurons showed a positive correlation between ROS formation and DNA damage. Our data obviously suggested that F exhibits genotoxicity by increasing DNA fragmentation. We can infer that 10 and 25 mM BA treatment resulted in a significant reduction in DNA fragmentation rate by preventing oxidative stress.

Conclusion

High concentrations of F exposure have been shown to cause severe oxidative stress-induced neurodegeneration. Experimental studies on the detection of neurodegenerative damage caused by fluorosis are increasing day by day. In this study, we found that BA has neuroprotective effects against cellular damage caused by fluoride. BA is taking significant steps towards becoming a new therapeutic agent, especially by giving positive results on neurodegenerative diseases. This fundamental study of the possible neuroprotective effect of boric acid against fluoride neurotoxicity will provide new perspectives for both researchers and clinicians to work towards the therapeutic use of boric acid. But there is needed more meticulously designed molecular studies regarding boric acid as an important protective agent against oxidative stress.

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Author's Contributions: CH, FK, HS, GK: Research concept and design, data collecting, analysis and interpretation of data. **CH:** Preparation of article and revisions. All authors approved the final version of the manuscript,

Ethical issues: All Authors declare originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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Henoch schönlein purpura in children: Clinical features and risk factors of renal involvement

Atiye Fedakar[1]*

Abstract

Objective: This study aimed to evaluate the clinical and laboratory features of children diagnosed with Henoch Schönlein purpura (HSP), risk factors of renal involvement and their effect on prognosis.

Methods: A total of 80 pediatric HSP patients (44 males and 36 females) between ages 2 to 13 (average age 7.68±3.09) admitted to the Pediatrics Clinic and follow-up cases from the Pediatric Rheumatology and Nephrology Clinic of the Istanbul Medeniyet University, Göztepe Training and Research Hospital, between April 1998 and June 2003 were enrolled for the study. In order to precisely evaluate glomerular and tubular function, urinary β 2 microglobulin, microalbumin and tubular reabsorption of phosphorus (TRP) were determined.

Results: A retrospective evaluation of the HSP patients showed that 26 (32.5%) had symptoms of renal impairment. In terms of renal function, 20 (25%) out of the 54 asymptomatic children initially subjected to routine renal tests had renal involvement. In terms of age, there was a significant difference ($p < 0.016$) in developing renal involvement between patients above 5 years old and those younger than 5 years.

Conclusion: It was therefore suggested that long-term follow-ups in addition to examinations such as routine kidney function tests, tubular reabsorption of phosphate (TRP) and microalbumin levels should be conducted in order to detect the early phase of renal damage.

Keywords: Henoch Schönlein purpura, risk factors, renal involvement

Introduction

Henoch-Schönlein Purpura (HSP) is the commonest small-vessel vasculitis in childhood with non-thrombocytopenic purpura, joint, gastrointestinal system (GIS), renal, genitourinary and central nervous system involvement as clinical features of the disease. Basically, it is characterized by the deposition of an immune complex containing immunoglobulin A (IgA) and complement part in the capillary wall (1,2). Despite its prevalence at every age in childhood, its incidence is twice more in boys than in girls and between the ages of 5 and 7 (3,4,5). Its annual incidence was reported as 10-22/100000. Although its etiopathogenesis is not exactly known, it has been reported that infections (bacterial, viral, parasitic), medicines, vaccines, tumors, bug bite and some foods may trigger the disease (6).

Although HSP is a benign self-limited disease, the most important factor determining its prognosis in the long term is renal involvement which is not manifested in all HSP patients. However, when renal involvement occurs, severe nephrotic proteinuria, macroscopic hematuria and kidney failure are the clinical manifestations (2,7). It is crucial to know the risk factors of renal involvement beforehand. In order to prevent the onset of complications, measures such as long-term follow-up of the patients should be conducted. To this end, this study aimed to present clinical and laboratory features of HSP patients following diagnosis at the Pediatric Clinic of Göztepe Training and Research Hospital, risk factors of renal involvement and their effect on prognosis in comparison with relevant literature.

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Material and Methods

A total of 80 HSP patients admitted to the Pediatrics Clinic (Internal Diseases) and follow-up cases from the Pediatric Rheumatology and Nephrology polyclinic of the Göztepe Training and Research Hospital, Istanbul Medeniyet University between April 1998 and June 2003 were enrolled for the study. The study was approved by the Ethics committee of the Göztepe Training and Research Hospital. Informed written consent were obtained from parents/guardians of the children. HSP diagnosis was made according to the American College of Rheumatology (ACR) criteria (8). The clinical features of HSP are; 1. palpable purpura, 2. diagnosis at <20 years old, 3. stomachache, and 4. skin biopsy showing the presence of leukocytoclastic vasculitis.

In the study, factors such as age, gender, complaints, drug intake and history of infection in the patients were determined. Secondary data were obtained from patient's medical records such as gender, age, clinical features of the skin, joint, kidney, GIS and other organs/systems. In patients with severe stomachache and/or positive fecal occult blood, GIS involvement was detected. During follow-up in patients with hematuria (proteinuria, edema, hypertension and decreased glomerular filtration rate (GFR), renal involvement was detected.

From the retrospective study, complete blood count, antistreptolysin O antibody (ASO), serum c-reactive protein (CRP), complement C3, rheumatoid factor (RF), antinuclear antibody (ANA), sedimentation, throat culture, immunoglobulins, fecal occult blood, skin biopsy findings, complete urinalysis, creatinine, Na, K, Ca, P, GFH levels and renal ultrasonography were determined.

Patients whose renal functions were examined after 2.5±0.5 mean years of follow-up and diagnosed with HSP were called-in for a check-up and renal function, TRP, β-2 microglobulin and microalbumi levels were evaluated. In the evaluation of renal functions, in

The control group for the study included 20 health children comprising 11 males and 9 females aged between 3-17 years (average age 10.5±4.6).

In this study, blood and urine, urea, creatinine, Na, K, Ca, P analysis were carried out with Auto analyzer (Olympus AU-5200, Japan) at the biochemistry laboratory of the hospital while β-2 microglobulin and microalbumin in 24-hour urine test were analyzed using the Turbitimer method with Cobas Mira (Roche Diagnostic Systems Incorp., USA) in the Sonomed biochemistry laboratory.

Data obtained were analyzed using SPSS (Statistical Package for Social Sciences) software. Mann-Whitney U and Kruskal –Wallis tests were used for data comparison. In the Kruskal –Wallis and Mann-Whitney U tests, P values of <0.05 and <0.016, respectively were considered as significant.

Results

Of the patients included in the study were, 44 males (55%) and 36 females (45%); that is, male:female ratio of 1.22. Mean hospital length of stay was 6.63±4.4 days. 75% of the patients were >5 years old (mean 7.68±3.09). 26 patients (32.5%) had upper respiratory tract infection (URTI) and 4 (5%) had gastroenteritis with no history of vaccination and medication intake. When the seasonal distribution of the cases was examined according to their date of admission, the study showed that 29 (36.2%) cases were admitted in winter, 23 (28.7%) cases in spring, 29 (23.7%) cases in autumn, and 9 (11.2%) cases in summer.

The most common clinical features reported in the study were rash (80; 100%), arthralgia (47; 58.7%) and edema (30; 37.5%). Other symptoms included: stomachache, vomiting, arthritis, scrotal edema, headache, diarrhea, hepatomegaly and invagination. Elevated blood pressure of >95% percentile was reported (4 cases) in the study. Of the 80 cases, 3 cases were diagnosed with having only rash.

In terms of etiological assessment, the study showed increased ASO in 13 (16.2%) patients, mycoplasma in 2 (5%), Epstein barr virus in 2 (2.5%), Giardia intestinalis in 2 (2.5%), measles in 2 (2.5%), amebiasis in 2 (2.5%), group A beta streptococcus (GABS) in throat culture in 1 (1.2%), mumps in 1 (1.2%), leptospira in 1 (1.2%), and parvovirus infection in 1(1.2%) patient. Consequently, etiological cause was reported in 27 cases (33.7%). Fecal occult blood (FOB) was positive in 41 (51.2%) patients while GIS involvement was reported in 35 (85.3%) patients of >5 years.

A. Retrospective Evaluation of the Symptoms of Renal Involvement in the Patients with Henoch-Schönlein Purpura (With Routine Renal Function Tests)

When the findings of renal involvement were retrospectively evaluated from 80 patient-files, renal involvement was reported in 26 (32.5%) patients. Upon hospital admission, renal involvement was observed in 21 patients. However, within the first 3 months, renal involvement developed in 5 more patients (Table 1). Meanwhile, GIS involvement was found in 16 (61.5%) patients with renal involvement.

Due to post follow-up prolonged hematuria and proteinuria (3 months), renal biopsy was carried out on 3 males. The renal biopsy showed endocapillary proliferation of glomeruli with lesions close to 50% in 2 patients. However, in the third case, due to nephritic proteinuria and prolonged microscopic hematuria, there was mild mesangial cell proliferation in the biopsy.

An evaluation of findings from the first hospital admission of patients with HSP and laboratory results of those with renal involvement within 3 months and GIS involvement showed increased sedimentation ASO in the patients with GIS and renal involvement than the others.

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B. Prospective Evaluation of Renal Function in Patients with Henoch-Schönlein Purpura After 2.5 Years of Follow-up (with Routine Renal Function Tests, Microalbumin, β-2 Microglobulin, and TRP)

1. Evaluation of patients (Group I) on first admission to hospital with asymptomatic findings of renal involvement

When the cases were evaluated in terms of renal functions on average 2.5 years of follow-up after HSP diagnosis of, renal involvement was found in 20 (25%) of 54 patients initially evaluated as asymptomatic with routine renal tests (Table 2). GIS involvement was also found in 8 patients who were previously asymptomatic.

2. Evaluation of patients (Group II) whose findings of renal involvement were positive during their first admission to hospital

The 17 of 26 patients were found with renal involvement (65.3%) in their first hospital admission (Table 3).

The comparison between HSP patients with positive renal involvement after 2.5 years of follow-up and the healthy control group showed a significant difference between TRP and microalbumin/Cr levels ($p < 0.05$). However, there was no significant difference between β-2 microglobulin/Cr levels and the control group ($p > 0.05$) (Table 4).

The study also showed a significant difference in developing renal involvement among HSP patients ($p < 0.016$) between patients aged >5 years and <5 years. However, no significant difference was seen in in terms of gender ($p > 0.016$). When the relationship between HSP patients with and without renal involvement with GIS involvement was statistically examined, there was a significant difference between microalbumin/Cr levels in patients with renal involvement ($p < 0.016$). CRP and sedimentation increased moderately in 14 and 5 patients, respectively. Leukocytosis was reported in 27 patients (33.7%) while leukopoenia was found in 2 (2.5%). Serum complement (C3) level and other immunoglobulins were normal, antinuclear antibodies (ANA) and rheumatoid factor (RF) were negative while IgA increased in 1 case (430 mg/dl).

Table 1: Retrospective Evaluation of Renal Involvement Symptoms in Henoch-Schönlein Purpura Patients

Symptoms	N	%
Microscopic hematuria	11	13.7
Proteinuria	3	3.7
Hypertension + Microscopic hematuria	3	3.7
GFH reduction + Microscopic hematuria	3	3.7
Decrease in urine density	2	2.5
Proteinuria + Microscopic hematuria	2	2.5
Hypertension + proteinuria	1	1.2
Microscopic +macroscopic hematuria	1	1.2

GFH: Glomerular filtration rate, **N:** Number of patients = 29.

Table 2: Prospective evaluation of renal function in patients with asymptomatic renal involvement on first admission (Group I) to hospital

Renal function	N	%
Decreased TRP	10	12.5
İncreased Microalbumin / C ratio	7	8.4
Microscopic hematuria	2	2.5
Decreased TRP + Increased Microalbumin / C ratio	1	1.2

TRP: Tubular reabsorption of phosphorus, **Cr**: Creatinine, **N:** Number of patients = 20

Table 3: Evaluation of patients with positive renal involvement (Group II) at first admission to the hospital.

Renal function	N	%
Improvement	17	65.3
Increased Microalbumin / C ratio	3	7.6
Increased Microalbumin / C ratio + Decreased TRP	2	7.6
Microscopic hematuria	2	7.6
Decreased GFH + Increased Microalbumin / C ratio	1	3.8
Decreased GFH	1	3.8

GFH: Glomerular filtration rate, **TRP:** Tubular reabsorption of phosphorus, **N:** Number of patients = 26

Table 4: TRP, β-2 microglobulin/creatinine, microalbumin/creatinine levels of the healthy control group and HSP patients with renal involvement (after 1 -year follow-up)

Groups	TRP (%)	p	Microalbumin/Cr (µgr/gr)	P	β2 Microglobulın/Cr (µgr/gr)	p
HSP patients with renal involvement	68.1 ±4.8	0.045	1.7±0.89	0.01	3.23 ±2.8	0.60
Control group	82.45±5.75	<0.05	0.25±0.21	<0.05	2.77±1.65	p<0.05

HSP: Henoch Schönlein purpura, **TRP:** Tubular reabsorption of phosphorus **Cr**: Creatinine

Table 5: Comparison of literature with Henoch Schönlein Purpura (HSP) and laboratory with clinical characteristics of patients in the current study

Source no	*	9	10	11	12	13	14
No. of patients	80	535	151	168	212	162	186
Time of observation	2.5 years	6 years	15.6 months	6-66 months	0	6 months	16.9 months
Gender F/M	45/55	42/57	61/90	66/102	95/117	77/85	89/97
Age (mean years)	7.68	6.9	7.4	8.8	6.93	7.5	7.4
Season	Winter		Winter		Winter	Autumn	
Etiology (%)							
URTİ	32.5		21.8	44		58	
Drug	0					0	
Vaccine	0		0.1	2		0	
Gastroenteritis	5		1.3	6		0	
Affected system (%)							
Purpura	100	100	100	100	98.1	100	100
Joint	62.5	57,6	57.6	35	69.8	68.9	94
GİS	51.2	49,7	73.5	20	75	76.5	55
Renal involvement	32.5	49,9	27.1	20	26.9	56.2	28
İzole Hematuria(%)	13.7	5.2	25	59	9.9	24	6.9
Proteinuria (%)	3.7	77.5	16	35	1.9	2.5	2.7
Recurrence (%)	17.5		4	11.9	5.2		
Risk factors	>5year + GİS involvement	>6year + Atypical rash + occult blood in the stool	year	>7 year+ GİS involvement + long duration of rashes	>7 year+ GİS involvement	low albumin occult blood in the stool + diarrhea	>10 year +Female sex+> CRP

***:** Current study, **GİS:** Gastrointestinal system, **CRP:** Serum reactive protein, **URTİ:** Upper respiratory tract infection

Discussion

Although the clinical course is usually good in HSP, life-threatening complications rarely develop. Similar results have been reported in terms of epidemiology, clinical features, organ involvement, and prognosis of HSP in similar studies. Long-term prognosis of HSP is associated with kidney involvement, and while only microscopic hematuria was observed in some of patients, permanent kidney damage may develop in others (15).

In this study, the clinical features of HSP patients and risk factors of renal involvement were compared with 6 studies on HSP from different countries (Table 5). Along with the patient number in this study and from the 6 studies, a total of 1494 patients were obtained.

16, 17 and 18 reported that HSP is most commonly seen in male children of age 7.7 (). However, a few studies (19) reported that it occurs more in females.

In the current and retrospective studies, we reported a higher prevalence in males while the average age was in agreement with the literature.

In terms of seasonal distribution of HSP in the literature, patients presented more frequently in winter and autumn while some studies reported spring (20,21). The results of this study were similar to the literature as 36.2% patients presented in winter while 28.7% in spring. In the 6 studies we examined, 2 reportedwinter while 1 was in autumn; no season was stated in the other studies.

Although the etiology of HSP is not completely known, frequent respiratory tract infections have been implicated. For the first time in 1948, Gairdner showed that HSP developed in association with GABS infection. In the study, 50% of patients' throat culture tested positive for GABS infection (22). In a study of children with HSP by Al-Sheyyab et al., they reported increased ASO titer compared to the control group (23). Of the 6 studies we examined, there was respiratory tract infection varying between 22-58% in 3 of them. In our study, URTI was at the rate of 32.5%, and we found increased ASO in 13 patients (16.2%).

The possibility of HSP patients developing permanent impairment in renal functions, even if rarely, is a major source of concern for clinicians. In the literature, the incidence of renal involvement in HSP varies between 20-54% (24). This variation is assumed to arise from the differences in criteria determining renal involvement. The incidence of HSP nephritis is gradually increasing as the main cause of chronic kidney failure in the pediatric patient group and this rate is increasing towards 5-15% chronic kidney failure in children with HSP nephritis (25). Although the pathogenesis of HSP nephritis is not completely explained, it is reported that cellular and humoral immune dysfunction may be the cause (26).

Microscopic hematuria is the most common finding. However, HSP nephritis can manifest itself in a wide range varying from mild proteinuria or isolated microscopic hematuria that may last for a couple of weeks and improve spontaneously to rapidly progressive glomerulonephritis (27). In our study, hematuria was also the most common finding. In the 6 studies we examined, 5.2- 59% isolated hematuria was reported.

In the literature, it is emphasized that the most important factor determining HSP prognosis is the initial severity of renal symptoms (28). Previous studies have reported that chronic purpura, severe abdominal pain, older age, corticosteroid therapy, previous allergic condition, density and severity of renal symptoms and low serum coagulation factor XIII level significantly affect renal involvement (29,30). It is emphasized that renal symptoms are more severe especially in children at and above the age of 5 (31). Clear or obvious development of bloody stool is a risk factor of renal disorder (32). Renal involvement risk increases by 4 times in patients with abdominal pain and 7.5 times in patients with bloody stool (33). In our study and in the 6 studies examined, average age was above 5, and there was GIS involvement in 4 of the studies. Since our study showed GIS involvement in 85.3% and renal involvement in 80.4% HSP patients above 5 years, we suggest that these be followed for a longer time and more frequently. There was renal involvement in 20 patients initially asymptomatic and prospectively evaluated 2.5 years after diagnosis. However, GIS involvement was previously seen in 8 of them. Thus, asymptomatic GIS involvement can be a risk factor for patients in terms of renal involvement after years.

Similarly, in a 162-case series conducted by Kızıldağ, it was reported that patients with positive fecal occult blood presented with severe HSP nephritis and underwent kidney biopsy. BUN and creatinine levels were higher in this group of patients (13).

When the cases were evaluated in terms of renal functions 2.5 years after HSP diagnosis, 17 of 26 patients reported with renal involvement in the first 3-month period improved (65.3%). Renal involvement was found in 20 (25%) of the 54 patients who were previously evaluated as asymptomatic with routine renal tests. In the study on 66 HSP patients by Sönmez et al. at the end of a 3-year follow-up, there were minor urinary findings in 15 patients, active renal disease in 4 and renal failure in 1 patient (34). At the end of our 2.5-year follow-up, there was minor disorder in 25 and active renal disease in 4 patients.

In a 36-case study by Muller et al., 24-hour urinary N-acetyl-beta-D-glucosaminidase (NAG) and alpha-1-microglobulin were examined. When the levels in patients on their first hospital admission and after 1, 6 and 12 months were compared, tubular proteins increased especially in the early and late phases of HSP (35). However, in these studies, TRP, microalbumin and β-2 microglobulin were not examined in the evaluation of renal function. At the end of long-term follow-up, glomerular proteins increased in patients who initially had symptoms of renal involvement. However, tubular function was most affected in the patients developing symptoms of renal involvement at the end of follow-up. Urinary β-2 microglobulin levels were normal in all the patients in our study. Normal urinary β-2 microglobulin level is associated with the fact that β-2 microglobulin is an unstable substance.

Conclusion

Finally, in terms of prognosis, knowing the renal involvement incidence of HSP patients at the beginning and determining whether there is minimal glomerular and tubular damage in patients with asymptomatic renal involvement are crucial. Long-term follow-up of asymptomatic patients with and without renal involvement initially is required during their adulthood. It is suggested that for long-term follow-ups, it will be more useful to conduct examinations such as routine kidney function tests, TRP and microalbumin levels to detect the early phase of renal damage.

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Ethical issues: All Authors declare originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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Impact of lack of rehabilitation follow-up care on the functional level and autonomy of vascular hemiplegic patients at Kinshasa University clinics on homecoming

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Abstract

Objective: To evaluate the clinical and functional profile of vascular hemiplegia patients when deciding on their return home after hospitalization and evaluate its impact on their caregivers.

Material and Methods: This prospective descriptive study involved 60 patients. It was devoted to the evaluation, on the one hand of the functional level and the autonomy of the patients at the entrance and the decision of the return home, and on the other hand of the social charge felt by the caregivers of these patients.

Results: Out of 60 patients (66.7% of men and 33.3% of women, mean age of 62.93 ± 11.3 years), 93% of patients had a score of autonomy less than 60 at home , with no significant difference between the baseline score and the return to home score (p = 0.22), for an average length of stay of 25 ± 12 days. On the other hand, of 60 caregivers, between 72% and 73% of caregivers had a significant homework load, and this load was significant as function and independence levels were low (p = 0.001). Also, a significant increase in workload was observed at home compared to the hospital (p = 0.000).

Conclusion: We have emphasized the importance of an acceptable level of autonomy and multidisciplinary cooperation to allow patients to return to their homes in order to ensure a good home care and reduce the heavy burden of caregivers who have also need a better quality of life.

Keywords: Lack of Rehabilitation, Follow-up care, Vascular hemiplegic, return home,

Introduction

Stroke is a common, serious and disabling condition that is recognized as a major public health problem. Its incidence is increasing more and more [1,2].

In the Democratic Republic of Congo (DRC) hospital admissions for stroke rank first among the conditions treated in Internal Medicine in Kinshasa and in the Neurovascular Unit of the Neuro-Psychopathological Center [3,4].

As the world's population tends to grow older [5], and in view of the lack of an effective policy for prevention of cardiovascular risk factors in our country, it suggests a strong growth in the prevalence of cardiovascular disease. Despite the decline in stroke-related mortality, which is explained by improved treatment management and longer life expectancy, stroke patients are likely to experience a longer period of disability before death and heavy burden for society [6,7,8].

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Thus, stroke pose and pose over the next decades of management problems of a growing number of dependent patients, applicants for a significant amount of human, material and financial to the hospital and at home [9-11].

To do this, the care system must be well coordinated to ensure a good continuity of follow-up of these patients, because the stroke, apart from being an acute pathology, requiring care in extreme urgency, is also a chronic disease whose disabling sequelae lead many patients to attend the health system over a long period. Hence, the transition in the different stages of care of these patients must be well ensured in order to integrate them into the process of their empowerment.

In developed countries, about 30% of patients suffering a stroke made hospitalized in subacute care and rehabilitation (SSR) with the waning of their care in acute care [12,13,14]; and the decision to return home is made after a preliminary functional assessment, and an analysis of the home that will accommodate the patient. On the other hand, other patients are referred to a Reproductive Care and Rehabilitation Service (SRH) despite their functional level and autonomy.

In the Democratic Republic of Congo, there is a lack of transition in this pathway of care for stroke patients and a lack of multidisciplinary cooperation in their care; all patients return to their homes after hospitalization because there is no SSSR. This is what motivated us to conduct this study, with the goal of assessing the functional and autonomic level with which stroke patients return home, and its impact on the lives of their caregivers

Material and methods

Nature and period of study

This prospective descriptive study for one year was conducted during the period from January to December 2017.

Framework of the study

The Department of Cardiology and Intensive Care of the University Clinics of Kinshasa served as a framework for the realization of this.

Sample

This prospective study focused on 60 hemiplegic vascular patients hospitalized from January 2017 to December 2017. It was devoted on the one hand to functional evaluation and independence of patients at the entrance and exit of the hospital (homebound), and secondly to its impact on the social burden felt by the caregivers of these patients.

Included in the study:

• Patient therefore the diagnosis of stroke was confirmed by a CT scan and / or brain MRI;

• Hemodynamically stable patient;

• Patient for whom a functional assessment was made initially and at the close of the medical file;

• Patient with a permanent caregiver who agreed to participate in the study.

Data collection technique

Data was collected from patients, their caregivers and medical records. In patients, we collected data related to the functional parameters and independence of entry and exit of the hospital; among caregivers, we evaluated the burden felt by caregivers in supporting patients. Evaluations for these were done during hospitalization and one week after return home.

The following factors were studied: the age of the patients and their caregivers, the length of stay in hospital, the level of independence and functional start and return to home evaluated respectively by the Barthel scale and the motor index of Demeurisse, trunk stability by the Trunk control test and finally the workload of the caregivers by the Zarit scale.

Statistical analysis

We used Pearson's chi-square test to study the relationship between different study variables, with a statistical significance threshold of $p \leqslant 0.05$.

Ethical consideration

All hemiplegic subjects had consented in writing to participate in the study according to the Helsinki Declarations. The information collected from hemiplegic subjects was kept confidential.

Results

This study involved 60 patients, each with a natural caregiver: 40 patients (66.7%) and 20 women (33.3%) for patients, and 52 women (86.7%) versus 8 men (13). , 3%) for caregivers. The mean age of patients was 62.93 ± 11.3 years and that of caregivers 48.8 ± 11.6 years.

The mean hospital stay of these patients was 25 ± 12 days with extremes of 6 days and 60 days.

In terms of the level of autonomy of these patients at the end of hospitalization, our series shows that out of 60 patients evaluated, 56 (93.3%) returned home with an overall score of autonomy <60 (ie 63.3% with a score of 30 and 30% with a score of 30/60); and there was no significant difference between the initial level of autonomy and the decision to return home (p = 0.22) (Table 1).

As for the assessment of trunk stability, more than half of these patients (56.7%) returned home with either low trunk stability (40%) or zero stability (16.7%). However, there was not always a statistically significant difference (p = 0.24) between baseline and return home status (Table 2).

The evaluation of motor level (upper and lower) by the motor index of Demeurisse according to the period of hospitalization did not show a statistically significant improvement until the return home (p = 0.25). In addition, the majority of patients (66.6%) were discharged from the hospital either with low motor activity (43.3%) or with nil motor activity (23.3%) (Table 3).

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Table 1: Evaluation and level of autonomy in returning home

Level of autonomy of barthel scale	Period of hospitalization			
	At the beginning of hospitalization		Upon return home	
	N	%	N	%
Total dependence (0 to 25/100)	46	76,7	38	63,3
Partial dependence (30 to 55/100)	14	23,3	18	30
Partial autonomy (60 to 85/100)	0	0,0	4	6,7

p=0.22

Table 2: Evaluation of trunk stability by Trunk control test

Trunk control test	Hospitalization period			
	At beginning of hospitalization		Return to home	
	N	%	N	%
No stability of the trunk (0 to 18/100)	18	30,0	10	16,7
Low stability of the trunk (19 to 38/100)	22	36,7	24	40,0
Stability of the trunk possible with (44 to 76/100)	20	33,3	20	33,3
Good stability (77 to 100/100)	0	0,0	6	10,0

p=0.24

Table 3: Evaluation of the level of motricity to limbs by the motor index of Demeurisse

Deumerisse motor index	At the beginning of hospitalization			
	At the beginning of Hospitalization		Return to home	
	N	%	N	%
No motor activity (0 to 9/100)	28	46,7	14	23,3
Low motor activity (10 to 39/100)	20	33,3	26	43,3
Average motor activity (40 to 59/100)	8	13,3	16	26,7
Acceptable motor activity (60 to 79/100)	4	6,7	4	6,7

p=0.25

Table 4: The burden of permanent caregivers of patients in hospital and home assessed by the Zarit scale

Scale of zarit	Period of hospitalization			
	At the hospital		At home	
	N	%	N	%
Light workload	20	33,3	4	6,7
Moderate workload	26	43,3	10	16,7
Large workload	14	23,3	46	76,6

p=0.000

Table 5: Influence of patients' level of autonomy on the workload felt by their caregivers

Zarit at home	Deumerise motor index								Total	
	No Motor Activity motricity		Low Motor Activity		Moderate Motor Activity		Aceptable Motor Activity			
	N	%	N	%	N	%	N	%	N	%
Light workload	0	0,0	0	0,0	0	0,0	6	10,0	6	10,0
Moderate workload	0	0,0	6	10,0	2	3,3	2	3,3	10	16,7
Large workload	4	6,7	26	43,3	14	23,3	0	0,0	44	73,3
Total	**4**	**6,7**	**32**	**53,3**	**16**	**26,7**	**8**	**13,3**	**60**	**100,0**

p=0.001

We assessed the burden felt by permanent caregivers in serving patients; thus, compared with the period of hospitalization, caregivers had a higher workload at home than at the hospital (76.7% vs. 23.3%). The differences were statistically significant (p = 0.000) between the hospital score and the home score (see Table 4). In addition, this workload of home caregivers was very significantly dependent on the level of motor activity (p = 0.001). Among 73.3% of caregivers who had a significant workload, 50% had either zero (6.7%) or low (43.3%) motor activity (see Table 5).

Discussion

The average age of patients was lower than reported in Europe [5,16] with male predominance seen in patients and female in caregivers. This lower average age in our patients can be explained by the fact that the European population is older than ours. In addition, caregivers were younger than patients and most often female. Also, the male sex is recognized as a risk factor for cerebrovascular accidents [17,18,19]. This was also found in our study where we observe a higher proportion of men.

The duration of hospitalization of these patients proved to be long; this may be related to the extent of the injury on the one hand, but on the other hand to the late payment of hospitalization bills by patients (families) who are extending their stay of hospitalization.

In assessing the state in which our patients were discharged from the hospital for a return home, it appears from this series that, relative to the level of autonomy, 93.3% of these were dependent in carrying out activities. of everyday life, with a score of Barthel <60. In addition, there was no significant difference between the level of autonomy of departure and that of return home (p = 0.22).

These results are totally contrary to what is reported in the literature [20,21,22]. Where it is said that the previous state, (measured by the Barthel's or Rankin's index), including age, as well as the severity of the stroke, are predictors of homecoming, and of becoming functional. This difference can be justified by the lack of transition of patients to follow-up care and rehabilitation before their return home in our environment.

As for the level of stability of the trunk, it was found that more than half of the patients returned home with either low stability of the trunk or with zero stability. Comparing the level of this trunk stability between the evaluation of the beginning of hospitalization and the return to home we did not observe a significant difference (p = 0.24). While poor trunk stability can impose a bedridden condition on the patient, which is logically associated with an obstacle to return home [23,24].

The evaluation of the level of motricity made by the motor index of Demeurisse showed no significant improvement (p = 0.25) before the decision of the return home. In addition, the majority of patients left the hospital either with low motor activity (overall score ⩽39 / 100) or with zero motor activity (an overall score of 0/100).

These results therefore show that most of these patients who left the hospital were not eligible for a return home, and this, given their level of autonomy, trunk stability and motor skills. Moreover, the decision of their return home was not dependent on the evolution of the level of autonomy, nor of the level of stability of the trunk, nor of the level of evolution of motor activity. These results are therefore contrary to what is reported in the literature, where the patient suffering from a stroke and hospitalized in a short-term service is eligible for a return home only with a score of autonomy ⩾60, and this, following a preliminary functional evaluation [22,25].

In the assessment of the workload (burden) felt by the caregivers of these patients, there was a significant increase in this at home compared to the hospital (p = 0.000). This big difference between the hospital and the home can be explained by the fact that in the hospital, the nursing staff compensates for some of the help given to the patients, whereas at home the patient is not presence only of his family. On the other hand, the home monitoring service does not exist in our community.

In addition, more than half of caregivers had a significant workload in helping their patients; whereas in a series described by A. Gallien it was found that the help of a family member was only necessary in 25 cases / 67, ie 37.3% (15). This difference is justified by the fact that in developed countries post-hospitalization patients do not all return directly to their homes, but often make a transition to a rehabilitation service, where they improve their level of autonomy and thereby reduce their workload among their caregivers.

Thus, this workload experienced by informal caregivers was very much related to the various aspects evaluated, notably the motor index, trunk stability and the level of autonomy, because the more these factors were deleterious, the greater the workload was important. (p = 0.001). This is in line with the NZAKIMUENA [27] series, which found a correlation between burden score (caregiver workload) and the importance of sequelae after stroke. Indeed, according to this series, the greater the functional deficit evaluated by the Barthel index, the higher the burden score "Zarit" was high, thus the heavy burden [26,28].

Conclusion

We have noted in this series that the vast majority of patients have left the hospital with a very low autonomy score, a motor function is zero or weak and poor stability of the trunk does not allow them to have a good maintenance and a good quality of life at home.

In addition, the decision to return home was not dependent on the functional status or level of autonomy of these patients. This situation has had repercussions on the permanent caregivers of these patients who have shown a very heavy workload, and thus a disruption of their quality of life.

Where did we find the need to create follow-up care and rehabilitation services (SSSR) in our community because they are non-existent.

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Pseudobulbar affect prevalence in Turkish multiple sclerosis patients

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Abstract

Objective: Pseudobulbar affect (PBA) is characterized by uncontrolled crying or laughing attacks which are usually socially inappropriate. The estimated prevalence in patients with multiple sclerosis (MS) ranges from 10% to 46.2%. We conducted a cross-sectional study to evaluate the prevalence of PBA in the Turkish MS population. Also, we aimed to estimate whether there was gender preference or coexistent depression.

Material and Methods: We used the Center for Neurologic Study - Lability Scale (CNS-LS) for this study. We included patients who were followed up at our outpatient clinic of Sultan Abdulhamid Han Education and Research Hospital with definitive diagnosis of MS at least for one year. The total number of patients was 328. 60.4% were women (198/328) and 39.6% were men (130/328). Descriptive statistical methods, student t test and chi-square tests were used for the analysis by using SPSS. The prevalence of PBA in the Turkish MS population was 39.6%. 34.6% of the men with MS had PBA; whereas 42.9% of the women with MS had PBA (p=0.132). The incidence of PBA was 48.1% in MS patients with depression and 38% in those without depression (p=0.175).

Results: As a result of t-test applied it was understood that depression did not significantly contribute to PBA frequency. The average depression test score was 13.28 in non-depressed, 17.85 in others. Furthermore, there was a difference between pathological laughing and pathological crying ($p<0.05$). Also, in both gender pathological laughing laughter was more and the difference was significant ($p<0.05$).

Conclusion: Our study revealed the increased frequency of PBA in MS patients. Gender and having depression did not make a significant difference on the PBA prevalence. However, depression significantly increased PBA test scores

Keywords: CNS-LS Scale, Gender, Multiple sclerosis, Pseudobulbar affect

Introduction

Sudden outbursts of involuntary, exaggerated laughter and/or crying have been described in patients with certain neurological disorders since the 19th century [1]. And, several different terms have been used for this clinical syndrome by clinicians such as “pathological laughing and weeping,” “emotional lability,” “pseudobulbar affect,” “emotional incontinence,” “pathologic emotionality” [2]. The term “pseudobulbar affect [PBA]” has generally been used more broadly, to refer to syndromes of exaggerated affective display which can be either mood incongruent or mood congruent [3,4]. Pseudobulbar affect [PBA] is characterized by uncontrolled crying or laughing which may be socially inappropriate to the social context. Thus, there is a disparity between the patient’s emotional expression and their emotional experience [5].

Despite the mechanisms are not fully understood, serotonergic and glutamatergic transmission is suggested to play major roles. Clinical improvements have been reported after treatment with SSRIs, TCAs or dextromethorphan/quinidine [5]. The underlying mechanism in PBA appears to be a lack of voluntary control, also termed cortical inhibition over brainstem centers that produced the facio-respiratory functions associated with laughing and crying. This loss of cerebral control results in a dissociation of affective displays from the subjectively experienced emotional states [6].

Detailed reviews of the widespread neuropathological and neurophysiological abnormalities found by neuroimaging and neurophysiological studies in patients with PBA have been published [7].

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PBA may coexist seen with amyotrophic lateral sclerosis [ALS], extrapyramidal and cerebellar disorders [Parkinson's disease, multiple system atrophy, progressive supranuclear palsy], multiple sclerosis [MS], traumatic brain injury, dementias like Alzheimer's disease, stroke, and brain tumors [8].

The patient's emotional response to a stimulant is often largely out of proportion . The crying or laughter may persist for a considerable period of time, and may not be suppressed by the patient. In addition, these episodes occasionally occur in situations that are not perceived by others as sad or being funny [5].

There is significant variability in reported prevalence rates, both between and within syndromes [1]. The range of estimates of prevalence in various neurological disorders is high, from 5% to well over 50%. This variability stems from the diagnostic criteria, methodologies, and patient populations studied [7-9]. Depending upon the scoring criteria used for the online instruments, prevalence rates ranged from 9.4%–37.5%, resulting in an estimated 1.8–7.1 million affected individuals in the USA [5]. PBA in MS patients is associated with severe intellectual deterioration, physical disability, and neurological disability [10].

The estimated prevalence of PBA in patients with MS ranges from 10% to 46.2% [11].Thus, we conducted this study to evaluate the prevalence of PBA in the Turkish MS population. In this direction, the prevalence of PBA in men and women was determined separately. Moreover, the prevalence of PBA with and without depression were investigated to determine whether depression is a vital factor of PBA.

Material and Methods

We included patients who were followed up at our outpatient clinic of Sultan Abdulhamid Han Education and Research Hospital with definitive diagnosis of MS at least for one year. The total number of participants was 328, 60.4% were women (198/328) and 39.6% were men (130/328). Depression was present in 15.85% (52/328) of MS patients participating in our study.

Patients with depression were identified by applying the Beck Depression Scale. The cut-off score for depression was 17 points. The Center for Neurologic Study - Lability Scale (CNS-LS) was used to determine the prevalence of pseudobulbar affectation (PBA). The Center for Neurologic Study – Lability Scale (CNS-LS) is a seven-item self-administered questionnaire that has questions regarding the control of laughter and crying, and has been validated in patients with ALS and MS.12 The responses are graded from 1 to 5 for each question, with the total score range from 7 (no excess emotional lability) to 35 (severe excess emotional lability). Patients whose scores were 15 or more score were considered to have PBA [11].

In order to determine the PBA prevalence and test results, descriptive statistical methods such as mean, standard deviation and percentage were used. Student t test and chi-square test were also used by SPSS in comparison between sex and depression. The findings were evaluated at 95% confidence interval and p <0.05 significance level. P values less than 0.05 were considered to have significant differences.

Results

PBA Prevalence in MS Population

Of the 328 MS patients participated in our study, 39.6% had PBA. The frequency of PBA was 34.6% (45) in men and 42.9% in women (85). There was no statistically significant difference between gender groups (p=0.132) (Table 1).

Table 1: PBA prevalence in MS population

Group	n	n%	Chi-square	df	p
Female	85	42.9	2.267	1	0.132
Male	45	34.6			

PBA Prevalence in Patients with and without Depression

PBA frequency was 48.1% (25) in those with depression and 38% (105) in those without depression. there was no difference between these groups (p=0.175). In addition, as a result of detailed analysis according to gender, PBA frequency was similar between male patients' groups whether they have depression or not (p=0.969). Women with depression seemed to have PBA more 56.3% than men. However, PBA frequency did not differ among women regarding depression (p=0.096) (Table 2).

Table 2: PBA prevalence in patients with and without depression

Group	n	n%	Chi-square	df	p
Depressed	25	48.10	1.841	1	0.175
Non-depressive	105	38.00			
Depressed Male	7	35.00	0.002	1	0.969
Non-Depressive Male	38	34.50			
Depressed Female	18	56.30	2.764	1	0.096
Non-depressive Female	67	40.40			

Test Scores in MS Patients with and without Depression

In patients with depression, the independent samples t-test total score was 17.85±8.06. In patients without depression, the mean test score was 13.28±4.88.(p<0.001). Therefore, PBA scores of patients with depression were significantly higher (Table 3).

In addition, the mean PBA score was 16.15±8.20 in men with depression and 13.14±5.11 in men without depression. (t = -1,588, p = 0.127). In women, PBA score was 18.91±7.91 in those with depression and 13.37±4.74 in those without depression (t = -3.826, p = 0.001) (Table 3). It is possible to demonstrate the rates of PBA frequency according to gender and depression (Figure 1).

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Table 3. Test scores in MS patients with and without depression

Group	Min	Max.	±ss	t	df	p
Depressed	7	35	17.85±8.06	-3.952	58242	0.000
Non-depressive	7	33	13.28±4.88			
Depressed Male	7	35	16.15±8.20	-1.588	21759	0.127
Non-depressive Male	7	33	13.14±5.11			
Depressed Female	8	35	18.91±7.91	-3.826	35398	0.001
Non-depressive Female	7	31	13.37±4.74			

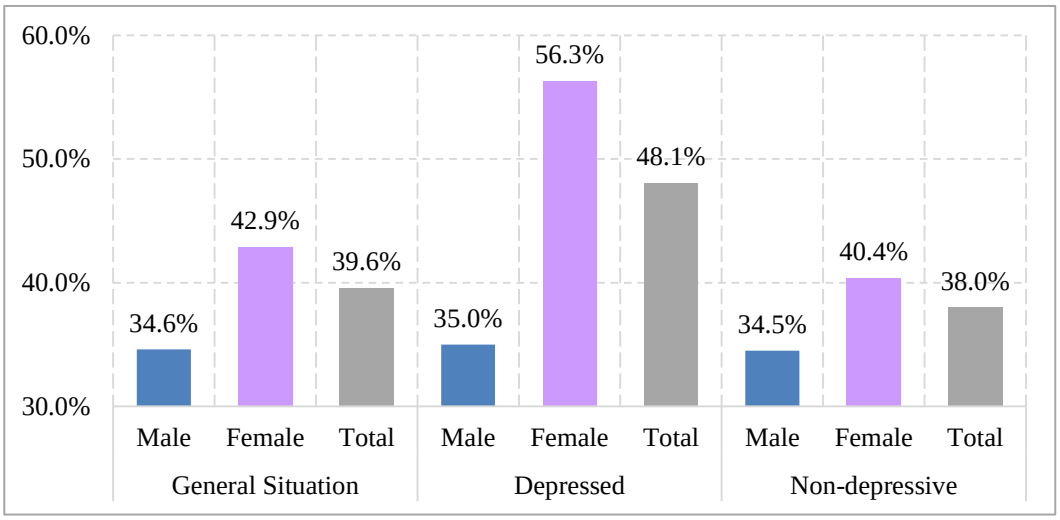

Figure 1. The rates of PBA prevalence

Item Levels

The item with the highest average among the CNS-LS scale items was "I find myself crying very easily" ($\bar{x}$=2.28). The item with the least average was "others have told me that I seem to become amused very easily or that I seem to become amused about things that aren't really funny", with $\bar{x}$=1.76. Accordingly, all items were at the "rarely agree" level (Table 4).

Table 4. CNS-LS scale item averages

Items	n		ss
• I find myself crying very easily	328	2.28	1.28
• There are times when I feel fine one minute, and then I'll become tearful the next over something small or for no reason at all	328	2.20	1.22
• There are times when I won't be thinking of anything happy or funny at all, but then I'll suddenly be overcome by funny or happy thoughts	328	2.11	1.15
• I find that even when I try to control my crying I am often unable to do so	328	1.97	1.17
• I find that even when I try to control my laughter I am often unable to do so	328	1.85	1.20
• I find that I am easily overcome by laughter	328	1.83	1.10
• Others have told me that I seem to become amused very easily or that I seem to become amused about things that aren't really funny	328	1.76	1.08

Table 5. Pathological crying and laughing scores

Score	Min	Max.	±ss	t	df	p
Pathological crying	3	15	6.45±3.11	-4.199	638943	0.000
Pathological laugh	4	20	7.55±3.62			
Pathological crying in Male	3	15	7.45±3.27	-3.228	248457	0.001
Pathological laugh in Male	4	20	8.92±3.99			
Pathological crying in Female	3	15	5.79±2.81	-2.960	394	0.003
Pathological laugh in Female	4	16	6.67±3.06			

Pathological Crying and Laughing Scores

The mean total score describing pathological crying (items 1, 3, 6) was 6.45±3.11; and the mean total score describing pathological laughing (items 2, 4, 5 and 7) was 7.55±3.62. The difference between the mean scores was statistically significant (t = -4.199, p < 0.001). (Table 5).

The mean pathological crying score in men was 7.45±3.27; and the average score of laughing was 8.92±3.99 (t = -3.228; p = 0.001). On the other hand, in women, the mean pathological crying score was 5.79±2.81; and the average score of crying was 6.67±3.06. (t = -2.960, p = 0.003) (Table 5).

Discussion

PBA can be accompanied by many neurological disorders. Previous research shows that it can be seen in ALS, Alzheimer's disease, Parkinson's disease, especially healing stages of stroke and following traumatic brain injury [14,16]. The number of patients in the US is estimated to be around 2 million [18,19]. Several clinical trials have reported that PBA incidence in MS patients is 10- 42.6% [15]. Vidovic et al. found this rate to be 41.8%. The PRISM trial results showed that 46% of MS patients had PBA [14]. However, in our study, the frequency of PBA in the Turkish MS population was 39.6%. We hereby showed that PBA prevalence among MS patients in Turkey is higher than that in the US. The geographical locations of the countries in the world may be affecting this. Furthermore, the prevalence of PBA was 34.6% in men; 42.9% in females. However, there was no gender preference for PBA among MS patients.. The incidence of PBA was 48.10% in MS patients with depression and 38.0% in those without depression. The co-existence of depression was not associated with PBA frequency in our MS cohort.

Our study supports the existing literature on increased PBA in MS. Moreover, we demonstrate the higher PBA co-occurrence with depression, and its potential consequences. The disease may be confused with mood disorders such as depression and bipolar disorder. However, the differential diagnosis can be straightforward with improved scales. Thus, proper patient management could be achieved..

The PBA incidence in men with and without depression was 35% and 34.5%, respectively. On the other hand, the PBA prevalence in women with and without depression was 56.3% and 40.4%, respectively. The co-existence of depression did not affect PBA frequency in neither men nor women. However, it is noteworthy that PBA frequency in women with depression was significantly higher than men with depression, reaching up to 60%.

Although accompanying depression did not affect PBA frequency, it significantly increased test scores. The average PBA was 13.28 in non-depressed, 17.85 in others. The average PBA in women without depression was 13.37 and in women with depression was 18.91.. However, depression in men did not make a significant difference. For men without depression, the mean score was 13.14, for men with depression was 16.15. Furthermore, both laughing and crying scores were higher in men.

Amongst the drugs that have been tried for PBA treatment, dextromethorphan or quinidine has been the first drug to be approved by the FDA in 2008, with limited previous clinical data. No study has shown antidepressant drug effectiveness [20-23].

We evaluated the PBA frequency and depression among MS patients. But we did not gather information about patients' ongoing medical treatment including antidepressants or sedatives., We were unable to demonstrate an association between clinical parameters and underlying psychiatric disease or localization of demyelinated plaques. This may have been a confounding factor. .

Further studies investigating the clinical and radiological associations in MS patients with PBA would highlight the mechanisms of PBA pathology.

Conclusion

In conclusion, our study revealed the increased frequency of PBA in MS patients. PBA can cause anxiety and social inhibition which can effect patients' quality of life. Recognizing this challenging disorder may help clinicians improve patients' social functions.

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