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Effect of antioxidant rich diets on some autistic children in Egypt

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Abstract

This research was applied on two groups of autistic school children (6-10y). Group1 consumed experimental meals which had industrial products that containing preservatives snacks and cans in addition the experimental natural herbs and foods containing antioxidants. Group 2 consumed healthy diets containing antioxidants in natural herbs and foods according to the recommend dietary allowances supplemented and preventing all industrial foods containing preservatives snacks and cans for6 consecutive months. The experimental diets were analyzed that showed increase the content of the average intake of calories carbohydrate, fiber, protein, fat, cholesterol, vitamins and minerals compared with the ordinary diet consumed at the start of the experiment.

Anthropometric results indicated that body weight and body mass index (BMI) of the autistic children in group1 was significantly increased in contrary to group 2 at the end compared to the corresponding values at the start the study. Serum total calcium level was increased significantly but iron, lead and catalase enzyme levels were significantly reduced in groups 1 while serum hemoglobin and mercury were significant reducedin group 2 at the end compared to the values of the start.

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Serum ferritin, HDL-c, vitamins (C&E) and glutathione peroxidase activity were significantly increased but serum copper concentration was decreased while serum total cholesterol, triglyceride, LDL-c ionized calcium level were in a non-significant reduction after consuming the experimental diets to the both groups (1&2) at the end compared with the mean values at the start of the study. It could be concluded that antioxidants as natural food and herbs improved the health status of autistic children under investigation.

Key words: Autism children –antioxidant- healthy foods- herbs.

INTRODUCTION

Autism is a syndrome of mental disorder characterized by significant disorders in the features of the individual such as perception, regulation of behavior and emotion which revealed biological and psychological dysfunction in addition to mental performance developmental disorders that associated with the distress or social disability **(Lincoln et al., 2007and Happé 2015)**.

It has been shown that the promotion of a comprehensive normal diet with different antioxidants will lengthen the existence of the organism, improve their health, and reduce signs of aging**.** Antioxidants generally act as one combined group in antagonism to many types of free radicals in cells and in body in different ways as well. In addition, the combined effects of antioxidants are better than the effects of each antioxidant alone, and some antioxidants recover their effectiveness by other antioxidants **(Sarkar and Das 2006)**.

The naturally occurring antioxidants inside the cells are not enough, resulting in the production of a group of compounds acting as antioxidants called the antioxidants manufactured, some of which are added to foods to prevent the oxidation of their components of fat, sugars and proteins. The most common compound is butylatedhydroxytoluene (**Hu et al., 2006, Dawidowicz et al., 2006 and Peschel et al., 2006)**.

The antioxidants in food, especially vitamin A, C, E, zinc, selenium, manganese, copper, carotenoids, phenolic substances and flavonoids, as well as antioxidant enzymes play a role in the protection and

prevention of complications of autism children **(Uttara et al., 2009)**. There are some vegetables and fruits rich in such compounds as potatoes, radishes, cabbage, cauliflower, carrots, oranges, berries, grapes, apples and grapefruit. There are also some herbs that play the same role as cinnamon, cloves, thyme, nutmeg, anise and others. It reduces the free cracks in the body where the free radical to the formation of toxic substances affect the nervous system and lead to a defect in the centers of the brain and nerves, leading to the emergence of diseases including Parkinson's disease.Free amphibians take the electrons out of the healthy molecules in the body to neutralize themselves. This process, they may damage the cell membrane and the DNA material in healthy cells. It acts on the emergence of genetic mutations that act on the emergence of modern diseases, including cancer and autism, which informs the cell in the way it does its work, without a corresponding copy of this code carried by DNA **(Hey et al., 2000 and Scaglioni et al., 2011)**.

Antioxidants could prevent and or slow down the oxidative progression to maintain other compounds from free oxygen. Antioxidants in the body of the organism are found in the form of enzymes or co-enzymes or compounds containing reduced sulfur such as glutathione and naturally occurring antioxidants in vegetables, fruits, grains and most medicinal herbs. In recent years, the importance of antioxidants becomes greater than before due to its capability to protect the body from the invasion and elimination of bacteria and protects from incidence of the diseases. Recently, many researches proved the antioxidants effectiveness in the prevention of diseases, protection DNA damage, healing and returning of cells and tissues to normal activity **(Soobrattee et al., 2005, Dawidowicz et al., 2006, Al-Gadani et al ., 2009 and Cai et al., 2015)**. In this regard, these studies sought to illustrate the important nutrients rich in natural antioxidants and refrain from some foods so that affect the neurotransmitters that control the mood and reduces stress. Because of the increase in the prevalence of autistic patients and the inability to deal with these individuals, The present study was carried out to investigate the effect of consuming healthy diet that rich in natural antioxidant as in

vegetables and herbs as pumpkin, honey, white beans, carrots, radishes, cabbage, cauliflower, carrots, oranges, berries, grapes, apples, grapefruit, cinnamon, mint, anise, lemon cloves, thyme, nutmeg,gingerand rosemary on autistic primary school children.

MATERIALS AND METHODS

Materials:

Food and herbal drinks rich in the antioxidant nutrients were purchased from the local market. All Biochemical Kits and used chemicals were of analytical grade and procured from Sigma Chemicals Co., USA.

3- Methods:

A. Subjects and Design:

Thirty children between 6 -10 years old of primary school collected from the Institute of Graduate Studies for Children of Ain Shams University and some special centers for the treatment of autism in Maadi, Cairo - Egypt. Autistic children were classified into twogroups (15 autistic patients). Ethical guidelines were preserved during dealing with children while studying and permission was obtained from the Scientific Research Ethics Committee at Mansoura University. Children were fed diets supplement with the antioxidant-rich nutrients through 3 to 5 meals during the day as well as some natural tested herbal drinks. The experimental diets were taken over a period of 6 consecutive months.

The autistic children were divided into two groups as follows:

Group1: Autistic children had the experimental meals which contains industrial products that containing preservatives snacks and cans in addition the experimental natural herbs and foods containing antioxidants. Children had features as following:

Age	**7**	**6**	**8**	**8**	**7**	**7**	**7**	**8**	**7**	**8**	**8**	**7**	**8**	**6**	**8**
Tall	**132**	**104**	**111**	**124**	**122**	**127**	**131**	**142**	**117**	**129**	**131**	**126**	**133**	**109**	**121**
Sex	♂	♂	♂	♀	♀	♀	♀	♀	♂	♂	♂	♂	♂	♂	♂

Group 2: Autistic children were given healthy diets containing antioxidants in natural herbs and foods according to the recommend dietary allowances supplemented andpreventing allindustrial foods containing preservatives, snacks and cans.Children had features as following:

Age	**7**	٨	١٠	**8**	**9**	**9**	٨	١٠	٩	**10**	**6**	**8**	**8**	**10**	**10**
Tall	**129**	**137**	**140**	**135**	**136**	**138**	**139**	**142**	**145**	**144**	**127**	**132**	**132**	**150**	**155**
Sex	♂	♂	♀	♀	♂	♂	♂	♂	♂	♀	♂	♂	♂	♂	♂

- **Dietaryintake before the experiment:**

Children were questioned for their past 24-hour food intake for three days by household measures such as bowls, cups and spoon and then calculated in grams.

Average consumed diet by group 1:

Ordinary diet is composed of 100 gm chocolate cake, 300 gmFino bread, Mango Juice Can (200 ml),2 Large Chips Packet,50 g cooked cheese (Nesto),125g Luncheon, 200 gm burger, 60 g full fat white cheese, 200 ml whole milk, 15 g sugar and 50 g salted meat (pastrami).

Average consumed diet by group 2:

Ordinary diet is composed of big Packet Chips, Large molto, Manga juice can(200 ml), 200 ml whole milk, an egg, 200 gm cooked mallowkhia, 280 g Baladi bread, 250 gm fried chicken, 70 gm fried eggs with margarine, 60 gm Fino,40 g cooked cheese, 120 gm rice, 500 gmbanana and 25 g sugar.

- **Experimental Diets:**

Group 1 experimental diets:

The first group consumed ordinary diets that had industrial products containing preservatives as well as the use of dietary supplements besides the use of herbs and foods containing antioxidants according to recommended dietary allowance for 6 to 10 years old children. Dietswere illustrated as following:

At the first day:

The diet of first day is composed of a cup of half cream milk (200 ml), baladi brown bread (70 g), a piece of cheese Quraish (35 g), 75 g fresh lettuce,75 g fresh carrots, 75 g fresh radish, 75 g rice cooked with noodles, 100 g sautee mixed vegetables. 180 g boiled chicken without skin, a cup of half creamy yogurt, 85 g fresh orange fruit 60 g, 15 ml lemon juice and 25 g white honey.

At the second day:

The diet of second day is composed of a glass of orange juice (200 ml), 70 g brown brown bread, 80 g beans with oil and lemon, 35 g boiled egg, 500 g grilled tilapia, Sayadia cooked rice, 170 g half cup creamy yogurt,100 g of pumpkin, 15 ml lemon juice, 25 g white honey, a small cup of ginger (50 ml), 2 cups small cinnamon (150 ml), 2 cups anise (200 ml), 200 gm salad dish added 10 gm radish + 25 g avocado and 75 g fresh apple.

At the third day:

The diet of third day is composed of 2 cups half cream milk (400 ml), boiled egg, 70 g brown bread, 200 g grilled potatoes, 200 g grilled rosemary chicken without skin, 160 g rice with noodles, 130 g cooked spinach with sauce, 25 g white honey, Fresh orange fruit (60 g), 120 gm Kiwi, 15 ml lemon juice, a small cup of ginger (50 ml), 2 cups small cinnamon (150 ml), 2 cups anise (200 ml), 100 gm salad dish added 10 gm radish and 25 g avocado and 2 cups mint 200 ml (total 400 ml).

At the fourth day:

The diet of fourth day is composed of A cup of half cream milk (200 ml), a piece of chocolate cake (40 g), a cup of avocado juice (200 ml), Fino Loaf (60 g),50 g cooked cheese (Nesto), 150 g grilled chicken with rosemary without skin, 80 g pasta cooked with sauce,15 ml lemon juice, 25 g white honey, a small cup of ginger (50 ml), 2 cups small cinnamon (150 ml), 2 cups anise (200 ml), 100 g salad dish added 10 gm radish with 25 g avocado, 30 g red grapes, 2 cups mint 200 ml (total 400 ml), 75 g fresh apple and 30 g red grapes.

At the fifth day:

The diet of fifth day is composed of 140 g baladi brown bread, a glass of orange juice (200 ml), 75 g french fries,15 pieces stuffed cabbage with rice, 40 g turky cheese,150 g boiled chicken without skin, 15 ml lemon juice, 25 g white honey, a small cup of ginger (50 ml), 2 cups small cinnamon (150 ml), 2 cups anise (200 ml), 100 gm salad dish added 10 gm radish with 25 g avocado, 2 cups mint 200 ml (total 400 ml), 75 g fresh apple and 30 g red grapes.

At the sixth day:

The diet of sixth day is composed of a cup of yogurt 170 g, 35 g boiled eggs, 70 g brown bread, 30 g Quraish cheese, 120 g rice with noodles, 120 g cauliflower cooked with sauce,180 g veal meat, 130 gm kiwi, 40 g grapes, 50 g apple, 50 g orange ,15 ml lemon juice, 45 gm white honey, a small cup of ginger (50 ml), 2 cups small cinnamon (150 ml), 2 cups anise (200 ml), 200 gm salad dish added (10 gm radish with 25 g avocado) and 2 cups mint 200 ml (total 400 ml).

Group 2experimental diets:

Group 2: Autistic children consumed healthy antioxidant-fortified diets according to recommended dietary allowance for 6 to 10 years old children and prevent all industrial foods, cans and snacks that contain preservatives and supplements. Diets were illustrated as following:

At the first day:

The diet of first day is composed of 60g bread, ٥٠ g cooked cheese, cup of fresh grape fruit juice (200 ml), two cups mint (total400ml),٨ml lemon juice, 25g white honey, a cup of fresh avocado juice (200 ml), a small cup of ginger (50 ml), ٢cups small cinnamon (150 ml), ٢cup anise (200 ml), 75fresh apple, 30g red grapes,80 gm pumpkin, ١٢٠gm cooked rice with noodles,150gm cooked meat,110gm cooked spinach with sauce and salad dish (50g carrots,50 g cucumber, ٥٠g lettuce, 10 g radish and 25 g avocado).

At the second day:

The diet of second day is composed of 2 potato (200 g), 150 g cauliflower boiled with cumin, 140 g Baladibrown bread,30 g boiled eggs, fruit salad (50 g orange + 50 g grapes + 50 g avocado + 50 g apple),35 g white honey, a cup of half cream milk (200 ml),15 ml lemon juice, 2 cups mint (total 400 ml), A small cup of ginger (50 ml), 150 gm salad dish (10 gm radish + 25 g avocado),2 cups anise (200 ml), a cup of fresh orange juice (200 ml) and 2 small cups cinnamon (150 ml).

At the third day:

The diet of third day is composed of a cup of fresh avocado juice (200 ml), 60 gm plain cake, 250 g vegetable soup without potatoes plus 100 g cabbage, 200 g boiled chicken without skin, 120 g bread, 70 g boiled eggs, 40 g skimmed cheese, A cup of half cream milk (200 ml), 2 cups mint (total 400 ml). 15 ml lemon juice, 25 g white honey, a small cup of ginger (50 ml),2 cups small cinnamon (150 ml), 2 cups anise(200 ml), salad dish (50g carrots,50 g cucumber, ٥٠g lettuce, 10 g radish and 25 g avocado).

At the fourth day:

The diet of fourth day is composed of 120 g Fino, 90 gm beans with oil and lemon, 50 g fried potato, 20 cabbage rolls with rice, 150 g grilled chicken with rosemary, 30 g turky cheese, a cup of half creamy yogurt (125 g), A cup of grapefruit juice (200 ml), 30 g red grapes, 2 cups mint (total 400 ml), 15 ml lemon juice, 25 g white honey, a small cup of ginger (50 ml),2 cups small cinnamon (150 ml), 2 cups anise (200 ml),75 g fresh apple, 200 gm salad dish added with10 gm radish + 25 g avocado.

At the fifth day:

The diet of fifth day is composed of a cup of half cream milk (200 ml), a cup of yogurt half creamy (85 g), Fried graft tablet (25 g), Baladi bread (280g), 75 gm beans with oil and lemon, 30 g Quraish cheese, A cup of apple juice (200 ml), 400 gm grilled tilapia, 90 gm cooked white rice, 2 cups mint 200 ml total (400 ml), 15 ml lemon juice, 25 g white honey, A small cup of ginger 50 ml, 2 cups small cinnamon (150 ml), 2 cups anise

(200 ml), 200 gm salad dish added (10 gm radish + 25 g avocado), 75 g fresh apple and 30 g red grapes.

At the sixth day:

The diet of sixth day is composed of a cup of avocado juice (200 ml), 25 g full fat white cheese, 30 g boiled eggs,50 cooked Cheese, a cup of half cream milk (200 ml), 200 gm spinach cooked with sauce, 150 gm cooked veal, 50 gm rice cooked with noodles, 180 GmBaladi Brown Bread, Fresh orange fruit 60 g, 15 ml lemon juice, 15 ml lemon juice, 25 g white honey, a small cup of ginger (50 ml), 2 cups small cinnamon (150 ml), 2 cups anise 200 ml,200 gm salad dish added (10 gm radish + 25 g avocado), 75 g fresh apple,30 g red grapes and 2 cups mint 200 ml (total 400 ml).

B. Assessment of the Dietary intake:

The nutrients content of foods consumed by 24 hour recall (before experiment) and experimental diets were analyzing using a computerized nutrient analysis program. The values were computed to obtain the recommended dietary allowance (RDA) according to **Manore et al., (1989) and Whitney et al., (2002)**.

C. Anthropometric measurements:

Body mass index (BMI) was calculated {BMI = Weight in Kg / (Hight in meter)^2}. The grades of obesity utilizing the BMI were estimated as reported by **Garrow and webstar (1985).**

D. Analytical Methods:

Hemoglobin in blood and iron, ferritin cholesterol, triglyceride, low density lipoproteins cholesterol (LDL-C), very low density lipoprotein (VLDL-C), high density lipoproteins (HDL), vitamins(C & E), minerals (total calcium, ionized calcium, copper, lead, mercury) and activity of catalase and glutathione peroxidase enzymes in serum and also mercury in hair were estimated according to **Sandberg et al., (2015)**.

E. Statistical Analysis:

The obtained data was showed as mean ± SEM. Analysis of variance (ANOVA) test was applied to determine the significant among different groups according to **Armitage and Berry (1987)**.

RESULTS AND DISCUSSION

Table (1): Average intake of nutrients by autistic children groups

Variables Groups	Calories(Kcal)		Carbohydrate(g)		Fiber (g)		Total Protein (g)		Total Fat (g)		Cholesterol (mg)	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
Group1	661.43	3068.79	83.19	396.07	5.27	51.81	43.26	196.76	15.66	95.92	75.68	303.65
Group2	534.29	3238.1	67.81	375.47	6.60	62.8	33.44	172.18	14.37	100.6	28.47	267.08

Data in Table (1) showed that average intake of calories carbohydrate, fiber, protein, fat, cholesterol werehighafterconsuming the experimental dietsby group1 and group 2 autistic children. These results were explained by **Levy et al., (2007**) who reported that foods rich in antioxidants supplementation caused an increase in carbohydrate intake, these may be explained by of that body stores many of the nutrients absorbed from the intestine which are necessary for body functions.

The increasing the fiber intakecould improve the health status of children with autism.Dietsrich in fiber have numerous health effects on colon cancer, obesity, reducing risk of type 2 diabetes, cardiovascular disease and stroke (**Levy et al., 2007 and Bourassa et al., 2016)**. Regarding in this study, cholesterol taken from diets is useful for improving the health status of children with autism. Moreover, **Aneja and Tierney (2008)** suggested that cholesterol is important for myelin membranes growth, neuroactive steroid production, and normal fetal development. Also, cholesterol could change the ligand activity, G-protein coupling of the serotonin-1A receptor and oxytocin receptor. An insufficiency of cholesterol may contribute to autism spectrum disorders as reported in Smith-Lemli-Opitz syndrome (SLOS) and some subjects in the Autism Genetic Resource Exchange. Treatment of the individuals with

SLOS display with cholesterol could diminish infections, autistic behaviors, and symptoms of hyperactivity and irritability in addition of improvements in sleep, physical growth and social interactions. Moreover, cholesterol supplementation improved the self-injury, aggressive behaviors and temper outbursts. The single-gene disorder of abnormal cholesterol synthesis is a model for investigation of genetic causes of autism and the function of cholesterol in autism spectrum disorders. Cholesterol ought to be considered as a helpful treatment of ASD. **Wu et al., (2007) and de Souza et al., (2011)** reported that the influence of Nutrients and diets rich in omega-3 fatty acids have been shown to be essential in the upregulation of molecular mechanisms that maintain synaptic function and plasticity, deoxyribonucleic acid (DNA) synthesis, metabolism of hormones/neurotransmitters and aid cell proliferation.

Table (2): Some minerals intake of autistic children groups consumed experimental diets

Variables Groups	Calcium (mg)		Phosphorus (mg)		Total Iron(mg)		Potassium(mg)		Sodium (mg)		Zinc (mg)	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
Group1	1274.36	5190.90	294.00	1988.90	6.50	41.10	734.90	5686.00	665.50	2865.50	1.70	16.22
Group2	764.50	3309.86	299.30	2061.42	2.14	37.54	1000.20	6321.97	343.02	2350.68	2.57	19.84

Results in Table (2) illustrated the effect of experimental diets supplementation on minerals intake of autistic children groups. Datarevealed that as a result of introducing diets supplemented with some foods rich in antioxidants as well as herbs, the calcium, phosphorus,total iron, potassium, sodium, and zinc intake were increased significantly in all tested groups compared to the corresponding values at the start of the experiment.These results could be due to the beneficial effects of the antioxidants compounds in the diets supplementation.Many nutrients are essential for normal brain development, neuronal growth and function as zinc, selenium, iron, iodine, vitamin A, folate, choline, and long-chain polyunsaturated fatty acids. Poor nutrition during brain development in experimental animals is related to diminish in myelin production,

neurotransmitter systems and, brain cells and synapses **(Keunen et al., 2015)**.

Table (3): Vitamins (A,C,D&E) intake of autistic children groups consumedexperimental diets

Variables Groups	Vitamin A(µg)		Vitamin C(mg)		Vitamin D(µg)		Vitamin E(µg)	
	Start	End	Start	End	Start	End	Start	End
Group1	157.29	1160.3	25.34	80.72	1.04	3.32	2.70	17.94
Group2	102.46	718.00	34.50	143.50	0.07	2.59	2.81	18.6

Table (3) showed that the mean intake of vitamins (A, C, D, and E)were increased significantly after consuming experimental diets supplemented with antioxidants for all autistic children groups. It is known that exogenous chain breaking antioxidants like Vitamin E &C, and β-carotene could decrease the free-radical-mediated damages of neuronal cells and inhibit dementia pathogenesis in mammalian cells. The most important lipid-phase Vitamin E, is presented in lipid membranes and in the lipoproteins as low density lipoprotein, attenuate toxic effects of β-amyloid in neurological impairment and delay functional deterioration in autistic children **(Montiel et al., 2006 and Devore et al., 2010)**.

Table (4): Vitamins B intake of autistic children groups consumedexperimental diets

Variables Groups	Vitamin B1(mg)		Vitamin B2(mg)		Vitamin B3(mg)		Vitamin B6(µg)		VitaminB9 (µg)		Vitamin B12(µg)	
	Start	End	Start	End	Start	End	Start	Start	Start	End	Start	End
Group1	0.50	5.62	1.57	5.60	4.29	24.39	49.7	49.7	49.7	369.69	0.57	3.07
Group2	0.36	1.73	1.00	4.57	3.66	35.67	1.903	1.903	1.903	253.4	0.17	28.16

Table (4) showed results of vitamins B intake.Regarding toVitamin B1, Vitamin B2, Vitamin B3, Vitamin B6, Vitamin B9 and Vitamin12, total intake for these vitamins were increased after introducing the supplemented diets and herbs to the children. In recent times, Studies have proved that the essential nutrients such as trace elements, fatty acids,

amino acids, vitamins and minerals in have vital role in proper neural development and functioning of the central nervous system **(Nyaradi et al., 2013)**.

In summary, our results showed that the minerals and vitamins intake were increased after consuming the experimental diets. These results were similar to those obtained by **Hyman et al., (2012)** who found that the nutritional surveillance is essential for primary cares for all children including autism spectrum disorders children. That is needed to supply children with better nutrition, fortification of foods with vitamins and minerals to meet most required daily allowance. Dietary assessment with anthropometric and laboratory data are needs to a registered dietitian.Generally, choline, selenium, zinc, iodine, iron, folate, vitamins (A ,E and D) and long-chain polyunsaturated fatty acids are necessary for growth and functioning of the brain and also has been associated to the development of ASD **(Grant and Soles, 2009, Millichap and Yee 2012 and Surén et al., 2013)**.

Table (5): Means ±SD of Anthropometric measurements of autistic children groups consumed experimental diets

Variables Groups	Weight (k.g)		Height (cm)		Body Mass index (BMI)	
	Start	End	Start	End	Start	End
Group1	28.08 ± 2.55b	31.43 ± 2.90a	122.66 ± 1.33a	122.66 ± 1.22a	18.72± 0.23b	20.95 ± 0.23a
Group2	76.03 ± 5.77a	63.43 ± 4.05b	125.80 ± 1.53a	125.80 ± 1.34a	48.12 ±0.85a	40.12 ± 0.65b

Comparing values of the start with end, the different letters are significantly at P <0.05 and vice versa

The effect of diet supplementation on the anthropometric measurements of autistic children groups consumed experimental diets are shown in Table (5). Results indicated that body weight of the autistic children in group1 was significantly increased compared with their

corresponding values at the start of the study. On the other hand body weight of group 2 having the herbal drinks with their healthy diets showed significant reduction in the body weight.Concerning body mass index (BMI), results showed a significant elevation in group1and significant decrease in group 2 at the end of the experiment.

The autism spectrum disorders prevalence is raised and is affected one in 150 children. Children with autism spectrum disorders had a major health, behavioral and educational problems. Autism spectrum disorders appeared in different daily living like eating that may be picky or selective eaters. The experimental diets are rich in antioxidants as they contain ginger, onions, lemons, oranges and grapefruit,spinach and broccoli, ginger, cinnamon, green tea and turmeric and also had carotenoids, Vitamins (E,A,C&B), flavonoids, minerals which lower neurological disorders and diseases processes obtained from oxidative stress. The improvements in the obtained results were agreed with **Chauhan and Chauhan (2006)**. In addition, consumption of Curcumin and ginger that have phytoconstituents flavonoids act as mitochondrial antioxidant system powerful scavenger of the nitrogen dioxide, hydroxyl radicals and superoxide anion and protect also DNA against singlet oxygen–induced strand breaks **(El-Ghany et al., (2012)**. Also, these results may be explained on the basis of the nutrients absorbed from the intestine which are necessary for body functions. The natural antioxidant supplementation of the diet had good effects on body weight gain. These antioxidants played an important role in these results by supporting the increase of the body weight of the autistic children **(Meguid et al., 2017 and Egan et al., 2013)**. Nutritional supplementation with vitamin methyl-B12, folinic acid, and trimethylglycine is beneficial. One study in Romania found normal levels of vitamin B12 and folate in children with autism compared to controls, but low levels of plasma glutathione **(Paşca et al., 2009, and Li et al ., 2014)**.

Table (6): Means ±SD of ferritin, hemoglobin and iron of autistic children groups consumed experimental diets

Variables Groups	Ferritin (ng/ml)		Hemoglobin (g/dL)		Iron (m mol/L)	
	Start	End	Start	End	Start	End
Group1	14.80 ± 8.80^{b}	129.80 ± 10.60^{a}	7.15 ± 0.66^{b}	8.77 ± 1.22^{b}	20.8 ± 2.11^{a}	13.13 ± 1.40^{b}
Group2	136.50 ± 14.08^{b}	141.70 ± 19.80^{a}	$10.17 \pm 1.43^{*a}$	7.22 ± 0.78^{b}	17.53 ± 1.79^{a}	17.33 ± 1.33^{a}

Comparing values of the start with end, the different letters are significantly at $P < 0.05$ and vice versa

The present results (Table 6) demonstrated that serum ferritin levels was increased significantly after administered the experimental diets to the tested groups (1&2)however, hemoglobin was in a non-significant increase in group 1 and significant reduction in group 2at the end compared to the values of the start. Iron level in the blood of autistic children group 1 exhibited significant reduction at the end compared to the value of the start while group 2 showed a non-significant reduction at the end compared to the value of the start.

These results are confirmed with the study done by **Hergüner et al., (2012)** whostatedthat iron deficiency and anemia are common in children with autistic disorder. Thus, level of ferritin should be estimated in subjects with autism as a part of regular analysis. Another study by **Al-Ali and Alkaissi (2015)** indicated that there is an association between autism and nutrient intake as iron which related to anemia. Food selectivity is more widespread in children with autism compared to typically developing children. In another study by **Kita et al., (2011)** using10 boy with autism spectrum disorders, 11 healthy adult men and 13 normal developing boys. Oxygenated hemoglobin levels were increased in the area of the right inferior frontal gyrus compared to in the left inferior frontal gyrus. These activities mirror the severity of ASD, as the more serious ASD characteristics is correlated with decrease of activity levels.

Table (7): Means ±SD of serum cholesterol, triglyceride, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and HDL/ LDL of autistic children groups consumed experimental diets

Variables / Groups	Cholesterol (mg/dL)		Triglyceride (mg/dL)		HDL-c (mg/dL)		LDL-c(mg/dL)		VLDL-c(mg/dL)		LDLc /HDLc	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
Group1	160.3± 24.62a	138.4± 17.88a	79.86± 4.11a	75.6± 7.65a	36.47± 3.80 b	43.2± 4.90a	96.13± 5.66 a	84.73± 5.33 a	15.97± 1.45 a	15.12± 1.33 a	0.37± 0.01b	0.50± 0.09a
Group2	165.0± 23.50a	156.6± 20.78a	89.73± 7.09 a	88.2± 7.03a	37.8± 4.56 b	41.2± 4.60a	98.93± 7.80a	94.6± 8.23a	17.94± 1.08 a	17.64± 1.21a	0.38± 0.02b	0.43± 0.06a

Comparing values of the start with end, the different letters are significantly at P <0.05 and vice versa

Data in Table (7) showed a non-significant reduction in the serum total cholesterol,triglyceride,LDL-clevel at the end of the experiment for the tested children in groups1&2however, serum HDL-c concentration and the ratio of HDL/ LDL for the tested children (groups1&2)was increased at the endcompared with the mean values at the start of the study

These results are confirmed by **Kim et al., (2010)** reported that there was a significant correlation among autism, triglyceride and HDL-c level. 53 % of the plasma HDL-c variation was found in autism. The dyslipidemia in autism boys explained might be an indication of association between autism and lipid metabolism. In another study by **Schengrund et al., (2012)** they stated that cholesterol metabolism disturbance had been contributed to dementia, perhaps because of maintaining membrane fluidity and the integrity of lipid rafts.. Red blood cell (RBC) membranes isolated from children diagnosed with autism blood had significantly less cholesterol from control children. While cholesterol in the circulation could not permit brain barrier, a generalized defect in its synthesis that reflect on the concentration in the central nervous system and development of the behaviors associated with autism

Vancasse et al., (2001) confirmed that the plasma phospholipid fatty acids in the autistic subjects showed a marked lower at the levels of 22: 6n-3 (23%) in the autistic subjects resulted in (20%) significantly lower levels of total (n-3) polyunsaturated fatty acids without significant lower in the (n-6) PUFA series, and consequently a significant increase in the (n-6)/(n-3) ratio (25%). Concerning the increase HDL-c concentration in the blood, compared to the mentally retarded controls.In regarding to review of **Smith et al., (2002)** reported that VLDL-c concentration in serum revealed reduction in all tested groups when children consumed the diets rich in antioxidants, but this reduction was a non significantly. **Table (8): Means ±SD of serum vitamins C and E of autistic children groups consumed experimental diets**

Variables / Groups	Vitamin C (mg/dL)		Vitamin E (mg/dL)	
	Start	End	Start	End
Group1	0.59±0.01 b	0.97±0.02 *a	2.64±0.70 b	4.45±0.52 a
Group2	0.80±0.02*b	0.94±0.04 a	3.77±0.65*b	4.16±0.76 a

Comparing values of the start with end, the different letters are significantly at P <0.05 and vice versa

Table (8) showed that both vitamins C and E concentration in serum were increased significantly of autistic children groups (1&2) after ingestion of the experimental diets compared to the corresponding values at the start the study.

Deshpande et al., (2006) illustrated that vitamin C has been a calmative effect on behavior in humans. In animals, stereotyped behavior, one of the characteristics of autism, was reduced by treatment with vitamin C as affects in neurotransmitter called dopamine 6. Children with autism may have many troubles from free radicals that damage the brain and immune system. Vitamin C can protect the body against these damages as antioxidant. High doses of vitamin C intake by children with autism had significant helpful and treatment effects on behavior, sleep and gastrointestinal disturbance compared to the control in double-blind experiment. **Curtis and Patel (2008), Morris and Agin (2009) and**

Schmidt et al., (2014) indicated that vitamin E is a fat soluble and has antioxidant effect. Combination of omega-3 with vitamin E found to treat the speech disorder verbal apraxia. Verbal apraxia is a neurologically based motor planning speech disorder is a feature of autism spectrum disorders. Vitamin E established glutamate receptor and lower dexcitotoxicity related to high levels of glutamate. It is known that polyunsaturated fatty acids in the cell membrane are susceptible to lipid peroxidation and early destruction. Vitamin E and carnitine deficiency resulting from inflammation of the gastrointestinal tract and malabsorption, leads to neurological abnormalities. Many developmental disturbances as dyslexia, attention deficit hyperactivity disorder, apraxia and autism may be related with vitamin E and omega-3 fatty acid deficiency. The alterations in intake of vitamin and mineral nutrient may impact brain biochemistry, through their roles as coenzymes.

Table (9): Means ±SD of serum calcium, ionized calcium, copper lead and mercury of autistic children groups consumed experimental diets

Variables / Groups	Total Ca(mg/dL)		Ionized Ca(mg/dL)		Cu(mg/dL)		lead (mg/dL)		Mercury(ppm)	
	Start	End	Start	End	Start	End	Start	End	Start	End
Group1	8.32± 1.64 b	9.26± 1.99 a	3.58± 0.91 b	4.47± 0.93a	118± 12.93 a	87.53± 7.76 b	4.87± 0.44 a	3.25± 0.22b	2.05± 0.24 a	2.12± 0.11 a
Group2	7.84± 1.08 a	6.06± 1.03 b	3.79± 0.76 a	3.59± 0.88 a	110.5± 11.54 a	100.80± 9.22 b	2.98± 0.30 a	2.68± 0.36 a	4.78± 0.55 a	3.56± 0.31 b

Comparing values of the start with end, the different letters are significantly at P <0.05 and vice versa

Results in Table (9) revealed that when the serum total calcium level was increased significantly in groups 1 and significant reduction in group 2 at the end of the experiment. However, ionized calcium concentration in serum was innon significant increase both groups (1&2) at the end of the study. The copper concentration in serum for autistic children was decreased

significantly at the end of the experiment in both groups (1&2) while serum lead concentration was reduced significantly in groups 1 and a non significantly reduced in group 2but the concentration of mercury in serum was in a non significant increase in group 1and significantly reduced in group 2 at the end of the experimentcompared to the corresponding values at the start of the study.

Since calcium has a vital role in body, **Muldoon** e**t al., (2015**) reported that poke out the eyes of children with autism are calcium deficient so low calcium intake increase pain and poking out the eye. Calcium supplementation found to be effective in speech developed very quickly in children with autism. Low calcium supplementation during embryonic and brain growth could be a risk factor for unfavorable neurobehavioral outcomes so treatment with calcium blocked this behavior. Although parathyroid hormone, calcitonin and vitamin D were within normal levels calcium had decreased in urine.

Since ionized calcium has the vital role of brain, **Schmunk and Gargus (2013)** illustrated that rare variants in calcium, sodium and potassium channels and polymorphisms were linked subunits with susceptibility to ASD as schizophrenia, bipolar disorder and other neuropsychiatric disorders. In animal models with these genetic variations recapitulate endophenotypes correlates of autistic behavior seen in patients. An ion flux across the membrane regulates a variety of cell functions and has profound effects on brain functions.**Li et al., (2014)** reported that serum zinc level and Zn/Cu ratio were decreased in children with ASD and increased serum Cu levels compared with normal cases. Elevated copper levels are associated with infections, inflammation, trauma, Wilson's disease, excessive dietary intake systemic lupus erythematosus, as well as autism **(Yilmaz et al., 2005 and Chauhan et al., 2008)**. In regarding to lead and mercury results, **Yassa (2014)** who found that the main causes of autism are lead and mercury from environmental exposure and defect in heavy metal metabolism so chelating agents is needed to detoxify. Study on two groups of children with autism showed significant decrease in lead level

in the blood and mercury with the use of chelating agent (DMSA) beside the lower in the autistic symptoms. **Elamin and Al-Ayadhi (2014)** identified five industrial chemicals as developmental neurotoxicants based on epidemiological evidence that are toluene, lead, arsenic, methyl-mercury and polychlorinated biphenylsThere was a significant negative association between Zn/Cu and behavioral scores. The optimal cut-off value of serum levels of Zn/Cu which is an indicator for an auxiliary diagnosis of autism was projected to be 0.665 that gave way a sensitivity of 90.0% and a specificity of 91.7% based on the receiver operating characteristic curve. Also, vitamin and minerals play vital role in the synthesis of a number of these neurotransmitters such as serotonin and dopamine that are regulated by dietary fluctuations in the availability of their nutrient precursors. Alterations in brain levels of dopamine, serotonin, acetylcholine and γ-aminobutyric acid can happen with lowering in vitamins, minerals and trace elements. So that daily nutrient supplements including essential vitamins, minerals, specific amino acids and omega-3 fatty acids is effective in ASD management, due to their ability to modulate neurotransmitter levels **(Fernstrom et al., 2007, Nakamura et al., 2010 and Stewart et al., 2015)**.

.**Table (10): Means ±SD of serum catalase and glutathione peroxides of autistic children groups consumed experimental diets**

ariables Groups	Catalase (moL/L)		Glutathione Peroxides (U/gm)	
	Start	End	Start	End
Group1	4.14±0.30a	3.86±0.59b	0.96±0.80b	1.29±0.66a
Group2	0.87±0.005b	2.48±0.33a	0.40±010b	1.10±0.11a

Comparing values of the start with end, the different letters are significantly at P <0.05 and vice versa

Data in Table (10) revealed that catalase enzyme activity wasdecreased significantly in group1 but increased significantly in group 2 at the end of the experiment. However, concentration of glutathione

peroxidase activity in serum was significant increasein both groups because of introducing natural foods rich in antioxidants to the subjects.

Our results were agreed with those of **Abha and Ved (2006)** who studied the reactive oxygen species (ROS).and oxidative stress at the membrane in autism, measured lipid peroxidation and effectbof detoxifying in autism, elevation of lipid peroxidation markers represented high oxidative stress. Ceruloplasmin (copper-binding protein) and transferring (iron-binding protein) are major antioxidant serum proteins and decreased in children with autism. The alterations of them revealed to abnormal copper and iron metabolism in autism.Other researches had suggested that the antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase activities were altered in autism. Moreover, distorted glutathione levels and homo cysteine/methionine metabolism could increase in inflammation, excitotoxicity, and immune dysfunction **(Chauhan et al., 2006)**.

Chauhan et al., 2012 and Ghanizadeh et al., (2012) investigated the responsibility of oxidative stress and related factors that damage to the brain as well as oxidative stress reduction factors.In autism, sulfates level, methylation capacity and the total glutathione level were lowered while oxidized glutathione and the ratio of oxidized to reduced glutathione were higher in autism. On the other hand, glutathione peroxidase, superoxide dismutase and catalase were decreased. Autism is associated with shortage in glutathione antioxidant defense in selective regions of the brain. The turbulence in brain glutathione homeostasis may put in to immune dysfunction and apoptosis, oxidative stress mainly in the cerebellum and temporal lobe, and perhaps neuro-developmental abnormalities. The brain is extremely susceptible to oxidative stress because of lower antioxidant capacity, high amounts of unsaturated lipids and iron and high energy requirement. Glutathione is vital for neuronal survival, exists in the disulfide-oxidized and thiol-reduced forms and could detoxify and eliminate of free radicals and environmental toxins which damage the cellular functions through oxidizing proteins, lipids and DNA **(Franco and Cidlowski 2009)**.

It could be concluded that antioxidants as natural food and herbs improved the health status of autistic children under investigation so it is recommended toconsume natural foods and herbs that contain many antioxidants especially to autistic children.

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تأثير الوجبات الغذائية المضادة للأكسدة على بعض الأطفال المصابين بالتوحد في مصر

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الملخص العربي

أجريت الدراسة على مجموعتين من أطفال المدارس المصابين بالتوحد التي تتراوح أعمارهم من ٦ إلى ١٠ سنوات.المجموعة الأولي التي تتناول وجبات "الأطعمة السريعة "التي تحتوي على مواد مضافة صناعية وهي الوجبات الخفيفة وغيرها بالإضافة إلى الأعشاب الطبيعية التجريبية والأطعمة التي تحتوي على مواد طبيعية مضادات الأكسدة

والمجموعة الثانية التي تتناول وجبات صحية تحتوي على مضادات الأكسدة الطبيعية بجانب الأعشاب الطبيعية وفقًا للتوصية الموصى بها للوجبات الغذائية مع الامتناع عن جميع الأطعمة الصناعية التي تحتوي على المواد الحافظة المضافة وأجريت الدراسة لمدة ستة أشهر متتالية. وأسفرت نتاج تم تحليل الوجبات الغذائية التجريبية زيادة في متوسط محتووها من السعرات الحرارية و الكربوهيدرات والألياف والبروتين والدهون والكوليسترول والفيتامينات والأملاح المعدنية وذلك بالمقارنة مع الوجبات الغذائية العادية المتناولة في بداية التجربة.

وأسفرت النتائج الأنثروبومترية " المقاييس الجسمية " أن هناك زيادة معنوية في كل من وزن الجسم ومؤشر كتلة الجسم (BMI) من الأطفال المصابين بالتوحد في المجموعة الأولي عن المجموعة الثانية وذلك في نهاية الدراسة بالمقارنة بالقيم المقابلة في بداية الدراسة. كما كان هناك زيادة معنوية في مستوى الكالسيوم الكلي في سيرم الدم وانخفاض معنوي في مستويات إنزيم الحديد والرصاص والكاتالز في المجموعات الأولي بينما كانت نسبة الهيموجلوبين في الدم والزئبق في المجموعة الثانية منخفضة بشكل كبير مقارنةً بقيم البداية. كان هناك زيادة معنوية أيضا في كل من مستوي الفيريتين في سيرم الدم والليبوبروتينات مرتفعة الكثافة وفيتامين ج – ه ونشاط الجلوتاثيون بيروكسيديز وانخفاض معنوي في كل من مستوي سيرم الدم من النحاس و الكوليسترول الكلي والدهون الثلاثية والليبوبروتينات منخفضة الكثافة ومستوى الكالسيوم المتأين وذلك بعد تناول الوجبات الغذائية التجريبية لكل من المجموعة الأولي والثانية في انهاية الدراسة وذلك بالمقارنة مع متوسط القيم في بداية الدراسة.

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**** باحثة دكتوراه

ونستخلص من نتائج الدراسة أن المواد الغذائية الطبيعية والإعشاب المحتوية علي مضادات الأكسدة تعتبر غذاء طبيعي يساعد في تحسين الحالة الصحية للأطفال المصابين بالتوحد موضع الدراسة ولذلك وتوصي الدراسة بتدعيم وجبات ألأطفال المصابين بالتوحد بوجبات غذائية غنية بمضادات الأكسدة الطبيعية لتحسين حالتهم الصحية .

الكلمات المفتاحية: أطفال التوحد - مضادات الأكسدة - الأطعمة الصحية - الأعشاب.