Therapeutic Effect of Selected Plants on Autistic Rats

Department of Nutrition & Food Science, Faculty of Home Economics, Menoufa University, Egypt

Abstract

Introduction: Autism is a lifelong neurodevelopmental disorder impairs social reciprocity and communication. The incidence of autism has been rising at an alarming rate over the past three decades, although the prevalence seems to differ between countries. Aim: The aim of this Study is to investigate the effects of gluten-free casein free diet (GFCF) fortified with/out Sage (Salvia officinalis) or Ginkgo (Ginkgo Biloba) as a possible aid in the improvement of neurological, behavioral, neurochemical and histopathological changes associated with autism. Methods: A group of pregnant rats received a single intraperitoneal injection of 600 mg/kg Valproic acid (VPA) then their offspring were randomly distributed into 4 groups each 5 rats as following: group 1: fed with standard diet (VPA-SD) and served as control positive, group 2: fed with GFCF diet (VPA-GFCF diet), group 3: fed with GFCF diet plus Sage (VPA-GFCF+ Sage), and group 4: fed with GFCF diet (VPA-GFCF + Ginkgo), where five offspring from healthy pregnant rats (not injected with VPA), fed standard diet were served as control negative group. Therapeutic potential were measured using: the feed efficiency ratio (FER), serum level of (SOD), (T.A.C), (GSH), (MDA) and (5-HT) in addition to histopathological study of the cerebellum. Results: results showed that diet with Ginkgo significantly illustrated the highest effect on the FER compared to all other groups (p<0.05). There were mild differences in serum markers, where sage group expressed the highest effect followed by ginkgo and then the GFCF diet.
Histopathological of Cerebellum of rat from control positive showing necrosis, pyknosis and abnormal shape of Purkinje cells where Ginkgo group showing an improvements in necrosis and pyknosis. **Conclusions:** GFCF diets with herbal supplement demonstrated benefits effects in the treatment of disorders associated with autism.

**Keywords** : Autism, Gluten, Casein, Sage, Ginkgo

**Introduction**

Autism spectrum disorder (ASD) is a complicated neurological disorder with impairments within the social relationship [Maskiet et al., 2011](#). It considered as a multi-factorial disorder that is influenced by immunological, genetic and environmental factors. The prevalence lately increased with a ratio of 4:1 boys to girls, [Loomes et al., 2017](#). ASD children suffer from abnormal gluten and casein-related digestive enzymes and increased gut permeability. Without adequate levels of digestive enzymes, peptides resulting from gluten and casein fail to become amino acids in huge numbers. Increased gut permeability then allows the peptides to leak into the bloodstream, where they circulate and eventually cross the brain blood barrier [Elder et al., 2006; Mulloy et al., 2010](#). Adequate nutrition help to the relief, both digestive and metabolic as well as psychological changes [Meguid et al., 2017](#). The gluten-free/casein-free (GFCF) diet is a common dietary intervention for autistic children. The Diet Mechanism it is according to the opioid-excess theory [Compart and Laake, 2012](#).

The increased of oxidative stress have been reported in autism. This can be a common pathogenic mechanism in many major psychiatric disorders because the brain has a relatively greater vulnerability to oxidative damage. Oxidative stress can participate in the evolution and clinical manifestations of autism [Meguid et al., 2011](#).

Valproic acid (VPA) is an anti-epileptic drug and is used to treat manic-type bipolar disorder and migraine. The use of VPA through pregnancy is associated with a raised incidence of autism. Based on this observation, prenatal exposure to VPA has been used as a reliable animal model for autism. This model shows neuroanatomical, and biochemical results that summarize the main characteristics of autistic children [Bambini-Junior et al., 2011](#). The brain of autistic...
children shows several neuropathological abnormalities like reduced number of Purkinje cells in the cerebellum, while neurochemical, the most consistent finding in ASD is an increase in blood serotonin. Interestingly, serotonin is known to play an active role in brain development Abdelrahman, 2008.

Ginkgo biloba (Ginkgo Biloba) belonging to the Ginkgoaceae family. The active components include flavones, ginkgolides, and bilobalides Chen et al., 1998; Hauser et al., 2002. Flavonoid glycosides are antioxidants that can protect neurodegenerative diseases due to antioxidant stress Ramassamy, 2006. The extract has effects on improving blood flow to organs and tissues to protect against free radicals that are related to the nervous system disorders Smith et al., 1996. It is effective on the neurotransmitter system and on the antioxidant effect that related to the pathogenesis of ASD. Ginkgo Biloba, effective as an additional treatment of deviation in reciprocal social communication, verbal and non-verbal communication is the key to the characteristics of ASD Niederhofer, 2009; HasanZadeh et al., 2012.

Sage (Salvia officinalis) belongs to the mint family. There are sage types that studies suggest to help in the treatment of various diseases such as dementia, autism, lupus, cancer, heart disease, obesity, includes depression and diabetes Hamidpour et al., 2014. In addition to cholinergic activity, a wide range of activities has reported for the Salvia, these include tannic acid, oleic acid, ursolic acid, cornscole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavonoid glycosides, and estrogenic substances. It is used in heritage medicine for reducing oxidative stress and free radical damage Anamaria et al., 2013.

Material and Method:
Materials

This experiment was conducted using Albino Wistar rats at national animal welfare, the national research center, dokki, Egypt. Standard or basal diet was formulated to contain 14% casein, 10% sucrose, 5% corn oil, 5% fiber (cellulose), 3.5% mineral mixture, 1% vitamin mixture, 0.25% choline chloride, 0.3% D-L methionine, and
60.95% corn starch Reeves et al.,1993. GFCF was prepared by replacing gluten or casein with soy protein. VPA was purchased from (Sigma, St. Louis, MO). Dried Salvia officinalis was purchased from Environment Fund and the Community Service, Research and experiences of medicinal plants and aromatic Center, Faculty of Pharmacy, Cairo University, Egypt, Ginkgo biloba extract 260 mg/5ml was purchased from EIMC united pharmaceuticals for EMA pharm pharmaceuticals, Egypt.

**Experimental design**

The experiments were conducted according to national animal welfare standards and the ethics committee for institutional animals. On the 12th day of pregnancy, 20 pregnant Albino Wistar rats weighing 150-200g have been given a single intraperitoneal injection of 600 mg/kg VPA (using VPA dissolved in a saline pH 7.31 at concentration of 50mg/ml) Kim et al., 2013.

At the 21st of birth, the offspring male, were randomly distributed into 4 groups each 5 rats as following; group1: fed with standard diet (VPA-SD) and served as control positive, group 2: fed with GFCF diet (VPA-GFCF diet), group 3: fed with GFCF diet plus Sage (VPA-GFCF+ Sage), and group 4: fed with GFCF diet (VPA-GFCF + Ginkgo), where five offspring from healthy pregnant rats (injected only saline), fed standard diet were served as control negative group. Groups were given sage or ginkgo as fresh solution daily. Experiment was continued for 40 days. Therapeutic potential were measured using; the feed efficiency ratio (FER), serum level of (SOD), (T.A.C), (GSH), (MDA) and (5-HT) in addition to histopathological study of the cerebellum.

**Plant extraction and phenolic determination:**

The sage tea was routinely prepared by pouring 300 mL of boiling water with 4g of dried plant material and allowing steep for 5 min. Where Ginkgo extract 260 mg/5ml was adjusted to 300 ml with water. These solutions are given to rats instead of drinking water Tisserand & Young, 2013. Phenolic content was determined in water extract using Folin-Ciocalteu reagent and Gallic acid as a standard where Flavonoid content was determined Spectrophotometrically according to a standard method Quettier-Deleu et al., 2000.
Biological Evolution:  
During the experimental period (40 days), the diet consumed was recorded every day and body weight was recorded every week. The food efficiency ratio (FER), were determined according to Chapman et al., 1959. The catalase is determined according to Aebi, 1984. Superoxide dismutases (SOD) according to Nishikimi et al., 1972, malondialdehyde (MDA) is determined according to Ohkawa et al., 1979. Total antioxidant assay is determined according to Koracevic, et al., 2001, glutathione (GSH) determined according to Beutler et al., 1963.

Histopathology Examinations  
Small specimens of the organs brain were taken from each experimental animal, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70.80 and 90%), cleared in xylene and embedded in paraffin. Sections of 4-6 mm thicknesses were prepared and stained with hematoxylin and eosin according to Bancroft et al., 1996.

Statistical analysis:  
Data were expressed as Mean ±SD. T-test and analysis of Variance (ANOVA) between groups mean were calculated using the SPSS software for Windows Base, 2010.

Results  
Flavonoid and phenolic content of the sage and ginkgo illustrated in Figure (1). As shown Ginkgo was significantly higher (p≤0.05) in both flavonoid and phenolic content compared to sage.

The feed efficiency ratio Figure (2), rats of control negative group recorded higher FER in comparison with control positive group (p≤0.05). On the other hand, the rats of GFCF diet with/out herbal administrations showed a significant elevation in FER compared to the control negative group. FER of Ginkgogroup was the highest compared to all other groups (p ≤0.001).

Serum level of (SOD), (TAC), (GSH), (MDA ) and (5-HT) values from different treated groups are illustrated in Figure (3). As represented there was harmony between results obtained from different markers.
Control positive group showed a significant reduction of all markers compared to control negative group (p<0.05). On the other hand, a significant increase was noticed by the administration of the GFCF diet, Ginkgo and Sage. Diet with sage expressed the highest effect followed by ginkgo and then the GFCF diet.

Histopathological study of the cerebellum of different treated groups showed the Purkinje cell layer lying between the superficial molecular layer and a deep granular layer of Cerebellum. Cerebellum of rat from control negative (Figure 4a) showing no histopathological changes. Where Cerebellum of rat from control positive (Figure 4b) showing necrosis and pyknosis and abnormal shape of Purkinje cells as well as loss of Purkinje cells. Cerebellum of rat from GFCF diet group showing slight modulation in a number of necrosis of sporadic Purkinje cells compared to positive control. Cerebellum of rat from GFCF diet+sage group showing necrosis, pyknosis and atrophy of Purkinje cells and loss of Purkinje cells and chromatolysis. GFCF diet + ginkgo group showing improvements in necrosis and pyknosis of some Purkinje cells (Figure 4c-4e).

Figure (1): Flavonoid and phenolic contents of the sage and ginkgo. Data represented as mean of three replicates.
Figure (2): The Feed Efficiency Ratio (FER) of different groups. Data represented as mean ± SD.

Figure (3): Serum level of (SOD), (TAC), (GSH), (MDA) and (5-HT) in different treated groups. Data represented as mean of three replicates ± SD.
Fig. (1a): Cerebellum of rat from control- group showing no histopathological changes. Note normal Purkinje cells (H & E X 400).

Fig. (1b): Cerebellum of rat from control+group showing necrosis and pyknosis and abnormal shape of Purkinje cells and loss of Purkinje cells.

Fig. (1c): Cerebellum of rat from GFCF group showing necrosis of sporadic Purkinje cells.

Fig. (1d): Cerebellum of rat from GFCF+sage group showing necrosis, pyknosis and atrophy of Purkinje cells and loss of Purkinje cells and chromatolysis.

Fig. (4e): Cerebellum of rat from GFCF+ginko group showing necrosis and pyknosis of some Purkinje cells.

Figure (4): The histopathological changes indifferent treated groups.
Discussion

The present study confirmed the simulation of an experimentally induced animal model of ASD to the human one as regards neurochemical and histopathological parameters. It can be also used to study the effect of oral administration of plant extract on rats memory, concentration, mental alertness, and a decrease in mental fatigue Farooqui, 2013.

Rats fed GFCF diet exhibited a higher FER. These data are agreed with Whiteley et al., 2013 who suggested that the use of GFCF diet ameliorate symptoms, gastrointestinal disturbances, and improved developmental outcome. Moreover, when sage or ginkgo added to the diet the FER becomes higher. Ginkgo showed the highest effect on FER compared to all other groups. This date was agreed with Hasanzadeh et al., 2012 who reported an increment in food intake by 26% of autistic children receiving Ginkgo biloba added to their medication. Increased appetite may explain the high FER, especially in rats, received Ginkgo in their diet.

Patients with ASD and co-occurring gastrointestinal disturbances are at higher risk for oxidative stress. Oxidative stress in addition to other factors could contribute to the development and clinical manifestations of ASD Gorrindo et al., 2013. Elevated level of serum (SOD), (T.A.C), (GSH), (MDA) and (5-HT) reported in autistic patients as indication for oxidative stress. The pathogenic mechanism of psychiatric disorders implicated oxidative stress because of the high risk of brain damage through oxidative stress Annelies et al., 2018.

Data showed that sage followed by ginkgo were helpful in reducing the oxidative stress. Same results were reported by Hamidpour et al., 2014 who demonstrated that sage had a therapeutic effect in treating several diseases such as dementia, ASD, lupus, cancer, heart disorder, obesity, incorporates depression and diabetes Hasanzadeh et al., 2012. Ginkgo was effective on the neurotransmitter system and the antioxidant effect that may be related to the pathogenesis of ASD. Ginkgo also effective as a complementary treatment to the abnormality in reciprocal social communication, verbal and nonverbal communication Bahmani et al., 2016. Ginkgo extract exhibited its effect
through increasing the flow of blood to organ and tissue to protect from free radicals Marchezan et al., 2018.

Histopathology result of the present study confirmed that the most consistent neurological abnormalities in ASD is marked Purkinje cell loss in the cerebellum, Valko, 2007. The loss of Purkinje cells leads to social behavior deficits and increased and increased repetitive behaviors. Traditionally, the neurological basis of ASD has thought to lie mainly in the cerebral cortex Fatemit et al., 2012. The cerebellum has vast interconnections with the cerebral cortex and other parts of the brain, and evidence suggests that the cerebellum modulates and coordinates different functions throughout the brain Nadeem et al., 2019.

The brain in the early part of a development has a high risk of oxidative stress that's leading to the pathogenesis of neurodevelopment disorders and neuropsychiatric disorders similar to ASD Reith et al., 2013. Sage and ginkgo extract improve and protect Purkinje cell loss in the cerebellum by acting as potent antioxidants. Both extracts prevented the loss of Purkinje cells and retained the number and the shape of the cells. BistandBhatt, 2010; Dharet et al., 2018.

References
Anamaria, P.; Muste, S.; Mureșan, C.; Pop, C. and Salanță, L. (2013): Comparative study regarding the importance of sage (Salvia officinalis L.) in terms of antioxidant capacity and antimicrobial activities. Hop Med. Plants, 1(2), 41-42.


Gorrindo, P.; Lane, C. J.; Lee, E. B.; McLaughlin, B. and Levitt, P. (2013): Enrichment of elevated plasma F2t-isoprostane levels in individuals with autism who are stratified by presence of gastrointestinal dysfunction. PloS one, 8(7), e68444.


التأثير العلاجي لبعض النباتات على الفئران المصابة بالتوحد

عادل عبد المعطي احد، نهاد رشاد الطحبا، ساره عبدالوهاب الشريف،
埃及عبدالمجيد السعيدي.
قسمالتغذية وعلوم الأطعمة كلية الاقتصاد المنزلي، جامعة المنوفية، مصر.

الملخص العربي:

التوحد هو اضطراب عصبي معتمد يؤثر على السلوك والقدرة على التواصلاجتماعياً. قد زاد انتشار المرض بشكل ملحوظ خلال العقود الثلاث الماضية رغم اختلافنسب الانتشار إقليمياً. كان الهدف من هذا البحث هو دراسة مدى تأثير الوجبات الخالية منالكازين والجلوتين مع أو بدون أضافات النباتية أو الجبنية في تحسين حالة الفئران المصابة والتغيرات المرضية التشريحيه المرتبطة بالتوحد. تم حسن مجموعة من أناب الفئران الحياء بجرعة واحدة من محلول حمض الفوليك داخل الفئران البروتيني يتركز 1000 مجم/كجم، بعد أن يتم توزيع النسل عشوائياً إلى 4 مجموعات، لكل منها 5 فئران على النحو التالي:

- مجموعة 1: كونترول مؤجل تتغذى على وجبة قياسية، مجموعة 1: تتغذى على وجبة خالية من الكازين والجلوتين + مرية، مجموعة 3: تتغذى على وجبة خالية من الكازين والجلوتين + بركة أسود، مجموعة 4: تتغذى على وجبة خالية من الكازين والجلوتين + بركة أسود وحمض الفوليك، وتغذى على وجبة قياسية لاستخدامه كمجموعة مرجعية. تم تقديم التأثير العلاجي من خلال قياس معدل النمو، مستويات السيرم منحاولات، ومستويات السيرم منحاولات، ومستويات الدهون في المصل من الفئران في الملاحظات. أن النتائج أن للجبنية كلي اللمعانيد بالإضافة إلى التشريحي النسيجي للمخيخ، أظهرت النتائج السائدة لتفعيل تأثير معيق، ووضوح نتائج الفيزيكية لخلايا الفئران، وتفعيل الجبنية كلي اللمعانيد للجبنية. فعالة في تجنب الاضطرابات المرتبطة بالتوحد لدى فئران التجرب.

الكلمات المفتاحية: التوحد، الكازين، الجلوتين، المربدة، النباتية.