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Research Article

Pomegranate and Folate Ameliorate Isolation-Induced Autistic Like Behavior in Experimental Rat Model: Impact on Oxidative, Inflammatory, and Apoptotic Machineries

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Abstract

Recently more infants are prone to early separation from their mothers. Meanwhile, early life experience affects neurodevelopment and stress susceptibility. Maternal separation negatively impacts development causing different psychiatric and neurodevelopmental disorders like autism. In this study, maternal separation was done through separation of pups from their dams for [1.5 hr/day - 11 days]. Seeking protection, pomegranate (500 mg/Kg) and/or folic acid (50 mg/Kg) were ingested by dams during the lactation period (20 days). Maternal separation delayed pups' eye opening, impaired motor coordination decreased body weight and social activity in an open field arena as well as increased repetitive behavior in neonatal t-maze. Brain monoamine and reduced glutathione were decreased while nitric oxide, interleukin-1beta and caspase-3 were increased. Meanwhile intake of pomegranate and/or folic acid reversed maternal separation induced behavioral and biochemical changes. The combination protected against development of autistic-like symptoms in rats through modification of cells redox state and inflammation.

Keywords: *Autism; Maternal Separation; Pomegranate; Folic Acid; Apoptosis*

Abbreviations

ASD: Autism Spectrum Disorder; MS: Maternal Separation; POM: Pomegranate; FA: Folic Acid; PND: Postnatal Day; GSH: Reduced Glutathione; NO: Nitric Oxide; IL-1β: Interleukin-1 Beta; 5HT: Serotonin; NE: Norepinephrine; DA: Dopamine; NTs: Neurotransmitters; MAO: Monoamine Oxidase; ANOVA: Analysis of Variance

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental malady with basic symptoms of social and communication deficits as well as restricted and repetitive behaviors [1]. Epidemiological vigilance studies have demonstrated surge in pervasiveness of ASD in the United

States in recent years [2]. Besides, the rate of ASD diagnosis is higher in males [3]. Complex genetic as well as environmental risk factors (drugs, pollutants, stress, etc.) may be responsible for ASD development [1].

Infants are prone to stress through early separation from their mothers either due to decreased birth weight or low family income and the need for mother to go work. Regular maternal separation (MS) at the early postnatal stage is stressful to the neonates and may lead to alterations in many attentional, affective and emotional practices [4]. Meanwhile, early life MS is a generally used rodent model of early life suffering and as mentioned above; it is a liability factor for induction of neurodevelopmental disorders like ASD [5].

One of the suggested ASD mechanisms is immunological imbalance in the form of immune activation [6]. Meanwhile, immunomodulating and anti-inflammatory remedies such as prednisolone have shown some behavioral enhancements in open label preliminary tests and case studies of patients with ASD [7]. However, it is well documented that corticosteroids have significant adverse effects and they are likely to exacerbate behavioral symptoms in some subjects. At the same time, pomegranate (POM) extract, another immunomodulating agent, is safe with lower side effect profile. It has powerful anti-inflammatory and antioxidant activities [8]. It exerts immunomodulating action by decreasing pro-inflammatory (TNF-α, IL-1β) and increasing anti-inflammatory (IL-10) cytokines [9]. Moreover, POM has been largely used as neuroprotective agent for various degenerative diseases like cerebral ischemic/reperfusion brain injury [9].

Another major etiological component in ASD is oxidative stress. One third of children with ASD have markers for increased oxidative stress [10]. Meanwhile, it is suggested that impaired reduced glutathione (GSH) synthesis is evident in relation to the core social and behavioral sequences [10]. Antioxidants are crucial for neural survival during the early demanding period [11]. Folic acid (FA) has powerful antioxidant activity [12]. Additionally, adequate folate status is important during pregnancy and neurological growth in neonates and children [13].

Aim of the Study

The aim of the current work is to study the effect of POM and/or FA intake by lactating dams throughout the lactation period on pups' behavior, antioxidant and anti-inflammatory markers in MS induced autistic like symptoms in rat pups.

Materials and Methods

Animals

Adult male (200 - 220g) and female (140 - 160g) *Sprague-Dawley* rats, were obtained from the breeding colony of the National Organization for Drug Control and Research. They were kept in temperature controlled (25ºC) colony room on a 12/12h light/dark cycle and provided with their daily dietary requirements consisting of standard diet pellets and water was given ad-libitum. Ethics Committee of Faculty of Pharmacy, Al-Azhar University, Egypt approved the experimental protocol used in this study (No.167/2017).

Material

Pomegranate powder (91%) from whole fruit extract was purchased from Gongyi Xiangrui EcoMaterial Co. (Ltd, China). Folic acid was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Experimental design

Mating procedure

Female and male *Sprague-Dawley* rats were mated in the laboratory colony and the offspring served as subjects. Pregnant females were housed individually in plastic cages. Litters born before 3:00 pm were considered born on postnatal day (PND0). Within 24 hours of birth, rats were randomly chosen and were divided into five groups (4 - 6 neonate/mother and 16 - 18 neonates/group).

Maternal separation condition

Autistic-like symptoms were induced by MS of pups for (90min./day) and for 11 consecutive days [14]. Separation was done in a separate place in which temperature was hold at 28 - 30°C. During the isolation procedure, each pup was located in a separate cage with no bedding for 90 minutes. Isolated pup was able to hear and smell his mates, but not touch during separation. Autism was confirmed with reduced weight gain, delayed eye opening and impaired motor development as initial activities that manifest abnormal neuron development in pups [15].

Groups

Pups were separated from their dams in all groups except control group. Meanwhile, lactating mothers received either saline [control (C) and autistic (ISO)], Pomegranate (POM) (500 mg/Kg, orally) in 10% tween 80 in saline [9], Folic acid (FA) (50 mg/Kg, orally) in saline [16] or combination of them in the same doses. Treatments were received daily for 20 days. On PND21 male and female neonates were identified and only males were used for the experiment (females weren't affected in this model and that was identified from a pilot study; data not shown).

Evaluated parameters

Rat pups were evaluated for postnatal physical and neurobehavioral development as well as for functional behavioral deficits as illustrated in figure 1. Then, at PND46, rats were sacrificed by decapitation; Whole brain was carefully removed blotted and chilled. Brain monoamine contents were determined in 10% whole brain tissue after homogenization in ice-cold solution of acidified n-butanol. While oxidative stress biomarkers as well as interleukin-1 beta (IL-1β) contents were measured in 10% whole brain tissue after homogenization in cold saline that was centrifuged at 4000 r.p.m. for 15 minutes at 4°C. Additionally, brain expression of active caspase-3 was assessed from brains that were fixed in 10% formalin solution.

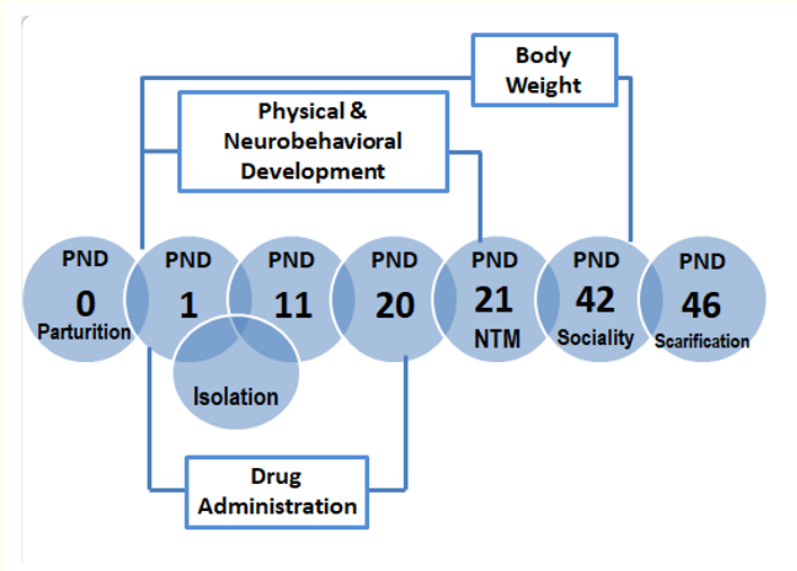

Figure 1: *Schematic diagram of experimental design.*

Physical and neurobehavioral development tests

Body weight changes

Rat pups were weighed at different ages before and after weaning (twice a week). Then, percent change in body weight from PND1 was calculated.

Development of physical signs

The test infant rats in all groups were daily examined carefully according to a suitable time schedule where the time at which the following physical signs had been evident were recorded for each rat pup in all groups [17,18]:

- **Pinna detachment:** Was observed until unfolding of the external ear was evident for each infant rat.
- **Downy hair:** Was observed until the appearance of primary coat of downy hair in all infants.
- **Incisor eruption:** Upper and lower incisors were observed daily until they had erupted through the gum-line for each test pup.
- **Fur development:** Was observed daily until complete development of fur for each infant rat.
- **Ear opening:** Was observed until complete opening of both ears for each neonate.
- **Eye opening:** Was observed daily for each pup until all test pups exhibited complete opening of both eyes.

Neurobehavioral development

Time of appearance of various reflexes and sensory functions were daily recorded for all examined rat pups in all groups according to a time schedule which determine the approximate time for development of these reflexes and sensory functions [17]:

- **Righting reflex:** Was observed until rat pup turns over to rest in normal position when placed on its back (from a supine to prone position), two trials per day with a maximum time allowance of 30 seconds per trial.
- **Cliff avoidance:** Was observed until infant rat crawls away from the edge of a cliff or a table top with the forepaws and face over the edge to avoid drop, it shows retraction and backward or sideward movement within 60 seconds.
- **Negative geotaxis 25˚:** Was observed until infant rat rotates up by 180˚ on 25˚ inclined plywood plane where placed in a head down position, pups were given one trial per day and allowed a maximum time of 60 seconds.
- **Palmar grasp:** Was observed until infant rat tries to grasp a paper clip with forepaws if stroked.
- **Negative geotaxis 45˚:** Was observed until infant rat rotates 180˚ on 45˚ inclined plywood plane from a head-down to head-up orientation; each infant was given one trial per day up to a limit of 60 seconds. It must be noticed that the approximate time for development of negative geotaxis behavior is directly proportional with the angle of the slope. Negative geotaxis is a test for motor coordination [19].
- **Auditory startle:** Was observed until a positive startle response (jerk of the head and extension of the hind limbs) is occurred to an auditory stimulus that lies 15 cm over the head of the infant rat, infants were given one trial per day and the presence or absence of startle response can be readily recognized.

Behavioral tests

T-maze spontaneous alternation test

This test was used as index of repetitive behavior. It was done at PND21. The NTM is made of black acrylic and measured 5.1 x 30.5 x 5.1 cm in the stem and 5.1 x 25.4 x 5.1 cm in the cross arm. The performance in the NTM was recorded as described by Vorhees 1983 and Chang., *et al.* 2017 [18,20] with slight modification. The test was performed under white light in a quiet room. At the end of the experiment, spontaneous alternation rate was calculated as the ratio between the alternating choices and total number of choices [20].

Social activity test

The test is based on the fact that when pairs of rodents of either the same or opposite sex are put together in a limited arena, they will engage in reciprocal social interactions [21]. The test was done at PND42 in an open arena that was described previously [22,23]. Pairs of weight-matched (±20g) rats, unfamiliar with each other and of the same treatment were placed simultaneously into the unfamiliar apparatus approximately 80 cm apart. Social behavior parameters were scored live for 10 minutes [21]. Time spent in active (sniffing, grooming, pinning and following) or passive (when animals lie next to each other within a distance of 5 cm from skin to skin) was scored for each rat.

The total SI time was calculated by the sum of the time spent in active and passive social behaviors during 10 minutes [21] observation period for each rat.

Biochemical tests

Estimation of brain monoamine contents

Serotonin (5HT), norepinephrine (NE) and dopamine (DA) were determined in brain tissue using the method of Ciarlone 1978 [24] while using spectro-photoflourometer RF-5000 Shimadzu, Japan. Calculations in the tested samples were determined as µg/ml wet tissue from the standard curve constructed.

Estimation of brain NO, GSH and IL-1β contents

Determination of brain nitric oxide (NO) was carried out according to the method of Montgomery and Dymock, 1961 [25] using a test reagent kit (Biodiagnostic, Egypt). While determination of brain GSH was done using a test reagent kit (Biodiagnostic, Egypt) and according to the method described by Beutler., *et al*. 1963 [26]. Both parameters were measured colorimetrically using Shimadzu double beam spectrophotometer.

Determination of IL-1 β was done using a test ELISA kit (Korain Biotech Co., China) and optical densities were measured using Biotek Elisa instrument. Calculations in the tested samples were determined as pg/ml wet tissue from the standard curve constructed.

Estimation of brain active caspase3 expression

Paraffinized brain sections were deparaffinized and rehydrated through xylene and alcohol for immunostaining, then 5 microns thick brain tissue sections were prepared. Immunohistochemical staining was conducted according to the manufacturer's protocol using Rabbit polyclonal Caspase-3 Antibody (active/cleaved) [Novus Biologicals (100-56113)] and secondary antibody [HRP Envision kit (DAKO)].

Statistical analysis

Data were expressed as means ± SE. The statistical significance was determined using One-Way Analysis of Variance (ANOVA) followed by Tukey as a *post-hoc* test. Percent change in body weight was analyzed using Two-Way Analysis Of Variance (ANOVA) followed by Bonferroni as a *post-hoc* test. Statistical significance was considered at $P < 0.05$. Statistical analysis was done using GRAPHPAD PRISM software (version 5, San Diego, CA, USA).

Results

Effects of pomegranate and/or folic acid on maternal separation induced changes in body weight

As illustrated in figure 2, isolation of rat pups significantly decreased percentage change in body weight. However, treatment of dams during the lactation period with POM or FA significantly increased percent change in body weight. Besides, combined POM and FA administration normalized percent change in body weight.

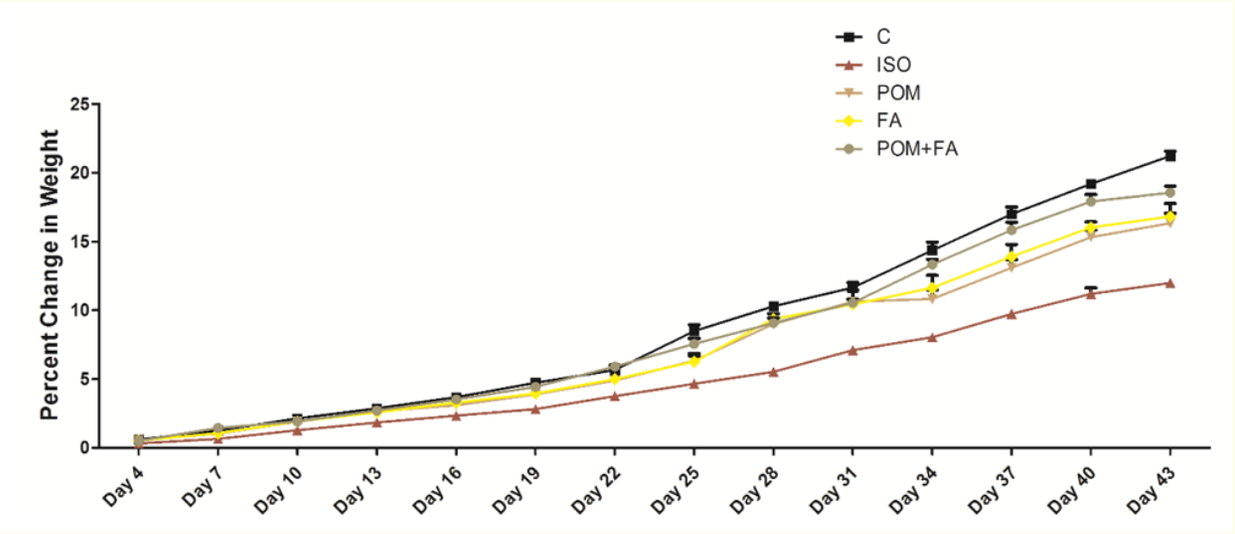

Figure 2: *Effects of pomegranate (POM), folic acid (FA) and their combination on maternal separation-induced (ISO) changes in the percent change in body weight. Values are means ± S.E.M. (n = 8 - 9). a: p < 0.05 compared with control; b: p < 0.05 compared with ISO; c: p < 0.05 compared with POM+FA.*

Effects of pomegranate and/or folic acid on maternal separation induced changes in physical signs development

As shown in table 1, isolation of rat pups significantly prolonged the time of eye opening to 111.06% as compared to control rats. On the other hand, there was no significant change in time of appearance of pinna detachment, downy hair, incisor eruption, fur development and ear opening in isolated pups as compared to controls. Treatment of lactating dams with POM, FA or their combination significantly restored pups' eye opening time to 90.83%, 92.49% and 94.02% respectively as compared to isolated rats. Also, there was no significant change in time of appearance of pinna detachment, downy hair, incisor eruption, fur development and ear opening in treated pups as compared to control and isolated pups.

Groups		Isolated (1.5 hrs/day - 11 day)			
Parameter	Control	ISO	POM	FA	POM + FA
Pinna detachment	3.68 ± 0.09	3.42 ± 0.21	3.19 ± 0.16	3.48 ± 0.12	3.44 ± 0.16
Downy hair	6.32 ± 0.11	6.30 ± 0.12	6.52 ± 0.11	6.20 ± 0.08	6.16 ± 0.07
Incisor eruption	10.25 ± 0.19	9.81 ± 0.28	9.80 ± 0.27	9.90 ± 0.23	10.67 ± 0.22
Fur development	9.86 ± 0.07	10.25 ± 0.09	10.23 ± 0.15	10.22 ± 0.09	10.10 ± 0.14
Ear opening	13.33 ± 0.13	13.37 ± 0.19	12.91 ± 0.15	13.41 ± 0.29	13.44 ± 0.18
Eye opening	13.56 ± 0.16	15.06^{a} ± 0.21	13.68^{b} ± 0.11	13.93^{b} ± 0.27	14.16^{b} ± 0.09

Table 1: *Effects of pomegranate (POM), folic acid (FA) and their combination on maternal separation-induced (ISO) changes in physical signs' development. Values are means ± S.E.M. (n = 8-9). a: p < 0.05 compared with control; b: p < 0.05 compared with ISO; c: p < 0.05 compared with POM+FA.*

Effects of pomegranate and/or folic acid on maternal separation induced changes in Neurobehavioral development

As illustrated in table 2, isolation of rat neonates significantly increased time of appearance of negative geotaxis 45° (i.e. impaired motor coordination) to 114% as compared to control pups. Meanwhile, there was no change in time of appearance of righting reflex, cliff avoidance, negative geotaxis 25°, palmer grasp and auditory startle as compared to control pups. Treatment of lactating dams with POM, FA or combination of both significantly ameliorated negative geotaxis 45° development time to 91.08%, 95.02% and 91.15% respectively as compared to isolated pups and the improvement in POM group made its value comparable to control one. Time of development of other reflexes and sensory functions including righting reflex, cliff avoidance, negative geotaxis 25°, palmer grasp and auditory startle weren't changed in treated rat pups as compared to control and isolated ones.

Groups		Isolated (1.5hrs/day-11day)			
Parameter	Control	ISO	POM	FA	POM + FA
Righting reflex	1.29 ± 0.11	1.35 ± 0.12	1.08 ± 0.08	1.19 ± 0.09	1.33 ± 0.09
Cliff avoidance	2.33 ± 0.09	2.42 ± 0.17	2.44 ± 0.16	2.50 ± 0.16	2.67 ± 0.11
Negative geotaxis 25°	9.52 ± 0.09	9.62 ± 0.13	9.30 ± 0.15	9.45 ± 0.18	9.61 ± 0.09
Palmer grasp	6.57 ± 0.11	6.76 ± 0.09	6.74 ± 0.10	6.50 ± 0.11	6.55 ± 0.11
Negative geotaxis 45°	11.93 ± 0.19	13.68^{a} ± 0.15	12.46^{b} ± 0.12	13.00^{ab} ± 0.17	12.47^{b} ± 0.10
Auditory startle	13.29 ± 0.13	13.40 ± 0.18	12.91 ± 0.15	13.39 ± 0.28	13.57 ± 0.19

Table 2: *Effects of pomegranate (POM), folic acid (FA) and their combination on maternal separation-induced (ISO) changes in neurobehavioral development. Values are means ± S.E.M. (n = 8 - 9). a: p < 0.05 compared with control; b: p < 0.05 compared with ISO; c: p < 0.05 compared with POM+FA.*

Effects of pomegranate and/or folic acid on maternal separation induced changes in behavioral performance

Spontaneous alternation in T-maze

As illustrated in figure 3, neonatal isolation significantly decreased pups' spontaneous alternation rate to 27.42% as compared to control. At the same time, spontaneous alternation was significantly increased after POM or FA treatment to 305.88% for both when compared to isolated pups while it was normalized (reached 364.70% of the isolated pups' value) after combined administration.

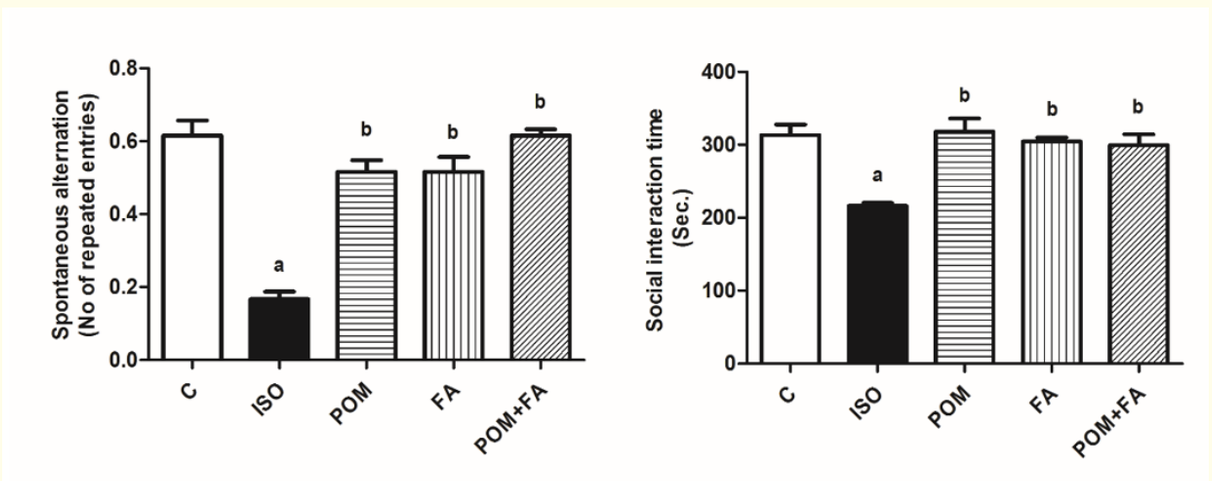

Figure 3: *Effects of pomegranate (POM), folic acid (FA) and their combination on maternal separation-induced (ISO) changes in the mean spontaneous alternation rate and social interaction time. Values are means ± S.E.M. (n = 8 - 9). a: p < 0.05 compared with control; b: p < 0.05 compared with ISO; c: p < 0.05 compared with POM+FA.*

Social interaction in open field arena

As shown in figure 3, neonatal isolation induced a significant decrease in social interaction time of pups' to 68.89% when compared to control rats. Pomegranate and/or FA intake significantly increased pups' social interaction time to 147.22%, 140.97% and 138.56% respectively when compared to isolated pups.

Effects of pomegranate and/or folic acid on maternal separation induced changes in brain monoamine contents

As illustrated in table 3, rat pups' isolation significantly decreased brain 5-HT, DA and NE contents to 69.29%, 81.25% and 84.97% respectively when compared to control rats. Lactating dams' treatment with POM or FA significantly increased rats' brain 5-HT content to 124.54% and 129.49% respectively as compared to isolated rats. Combining POM and FA resulted in normalization of rats' brain 5-HT content reaching 130.32% as compared to isolated pups. Treatment with POM and/or FA significantly increased brain DA content to 115.38% and brain NE content to 116%, 116.62% and 118.76% as compared to isolated rats.

Effects of pomegranate and/or folic acid on maternal separation induced changes in brain NO, GSH and IL-1β contents

As illustrated in figure 4, pups' isolation significantly increased NO brain content to 127.85% and IL-1β brain content to 124.17 as compared to control rats. Meanwhile, isolation decreased GSH brain content to 70.86% of the control value. Administration of POM and/or FA significantly decreased NO brain content to 71.18%, 70.25% and 65.89% respectively and IL-1β brain content to 80.43%, 81.68% and 66.99% respectively as compared to the isolated rats. Additionally, separate or combined administration of POM or FA significantly increased GSH brain content to 123.55%, 124.31% and 129.88% respectively as compared to the isolated rats.

Groups		**Isolated (1.5hrs/day-11day)**			
Parameter	**Control**	**ISO**	**POM**	**FA**	**POM + FA**
Serotonin content (µg/g wet tissue)	4.703 ± 0.165	3.259^{a} ± 0.100	4.059^{ab} ± 0.016	4.220^{ab} ± 0.163	4.247^{b} ± 0.044
Dopamine content (**µg**/g wet tissue)	0.016 ± 0.0002	0.013^{a} ± 0.0002	0.015^{b} ± 0.00007	0.015^{b} ± 0.0006	0.015^{b} ± 0.0005
Noradrenaline content (**µg**/g wet tissue)	13.24 ± 0.19	11.25^{a} ± 0.25	13.05^{b} ± 0.66	13.12^{b} ± 0.64	13.36^{b} ± 0.61

Table 3: *Effects of pomegranate (POM), folic acid (FA) and their combination on maternal separation-induced (ISO) changes in brain serotonin, dopamine and noradrenaline contents. Values are means ± S.E.M. (n = 6-9). a: p < 0.05 compared with control; b: p < 0.05 compared with ISO; c: p < 0.05 compared with POM+FA.*

Effects of pomegranate and/or folic acid on maternal separation induced changes in brain active caspase3 expression

As illustrated in figure 5, pups isolation resulted in strong expression of Caspase-3 in cerebral cortex, hippocampus, striatum and cerebellum. Cerebral cortex section of rats administered POM or FA showed moderate expression of Caspase-3. While in the striatum, mild expression was observed after POM administration and moderate expression after FA intake. However, combined administration of POM and FA weakened the expression of Caspase-3 in both cerebral cortex and striatum. In POM and combination groups, hippocampus and cerebellum showed weak expression of Caspase-3. While in FA group, mild expression was observed.

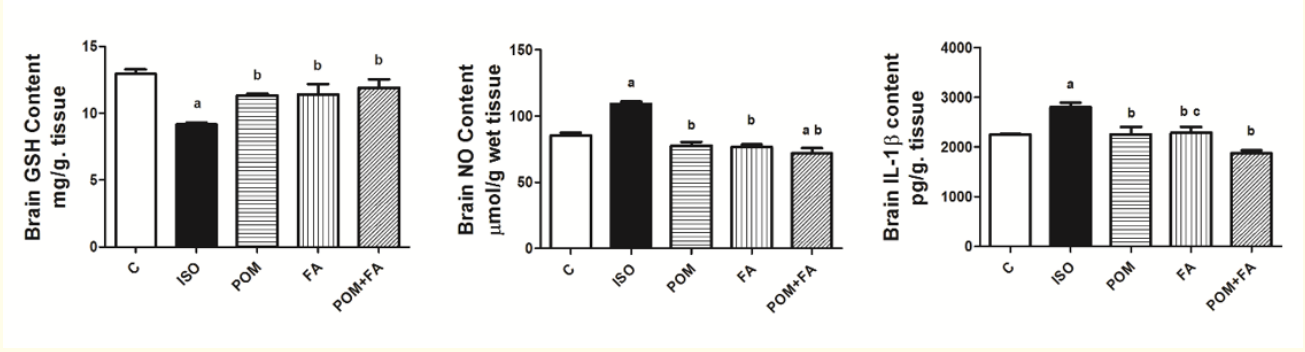

Figure 4: *Effects of pomegranate (POM), folic acid (FA) and their combination on maternal separation-induced (ISO) changes in the mean NO, GSH and IL-1β brain contents. Values are means ± S.E.M. (n = 6 - 9). a: p < 0.05 compared with control; b: p < 0.05 compared with ISO; c: p < 0.05 compared with POM+FA.*

Discussion

Autism is a neurodevelopmental disorder with specific behavioral and molecular/cellular hallmarks [1]. In this study, pups' MS delayed pups' maturation and caused autistic-like behavior in rat offspring. These results are similar to what was published about valproic acid administration which produced decline in weight gain, lag in eye opening time, impaired motor coordination and reduced social explorations [27]. Furthermore, studies have proven modified expression of cytokines and markers of redox state in blood, cerebrospinal fluid and brain in ASD individuals as well as in autism animal models [27,28]. Moreover, Sheikh., *et al*. [29] found that the expression of caspase-3 was elevated in the cerebellum of patients have autism. Meanwhile, MS caused decline in NTs content as described previously [30].

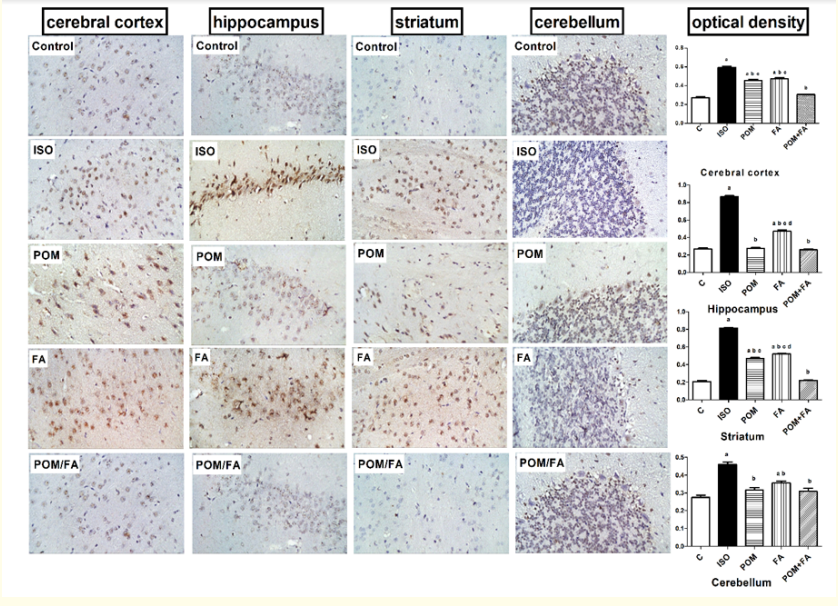

Figure 5: *Effects of pomegranate (POM), folic acid (FA) and their combination on maternal separation-induced (ISO) changes on active casp-3 expression in the cerebral cortex, hippocampus, striatum and cerebellum.*

Neurotransmitters (NTs) could control some of the behavioral impairments distinctive to ASD [31]. Decreased sociability and cognitive flexibility were observed in mice had 5-HT shortage [32]. Moreover, it was stated that DA is critical for diverse operations; it portray in working memory [33] and modify repetitive behavior activities [34]. Meanwhile the locus coeruleus-norepinephrine affect higher cognitive operations like attentional set shifting that is important in social activity [35].

Neurotransmitters signaling are regulated by mitochondria which offer ATP, mediate lipid and protein formation, and balance apoptotic and resilience pathways [36]. But in this study, MS induced oxidative stress which was evidenced by increment of NO and decrease of GSH. Increased oxidative stress causes mitochondrial dysfunction, glial cells activation and neuroinflammation [37]. So, shortage in NTs content may be related to increased oxidative stress. At the same time, oxidative stress induces cell death and this induction is dependent on the cell's capability to deal with it [38]. Even mild disruption in the main antioxidant, GSH, could have a huge outcome on brain development, particularly in the existence of precipitating environmental determinant like stress due to MS [39]. Upon occurrence of oxidative stress, microglia undergo activation and release proteolytic enzymes, complement proteins, NO and various cytokines [40]. Cytokines change normal neuronal function and behavior [40]. For example, IL-1beta exerts diverse effects on neural survival and proliferation as well as synapse development, migration, and differentiation. So high levels of IL-1β, brings about neuron loss or induces apoptosis [41].

In a trial to increase brain cells' antioxidant capacity in face of stress caused by MS; POM and FA were given to lactating dams. Pomegranate extract contains multiple bioactive agents that have antioxidant and anti-inflammatory activities and that are secreted in milk [42]. In the present experiment, isolated pups which received POM showed enhancements similar to that of other studies. Pomegranate enhanced weight gain decreased learning and memory defects and lowered anxiety [42]. These behavioral effects are linked to changes in NTs as well as oxidative and inflammatory status. It was suggested that POM's action was due to the tremendous content of polyphenols, mainly flavonoids, which are expected to decrease the monoamine oxidase (MAO)-A and -B activities in the brain and alter the monoamine levels [43]. Pomegranate extract improved redox state and attenuated apoptosis [44]. The reduction in caspase-3 by POM extract may be attributed to the antioxidant and anti-inflammatory activities of the active ingredients of the extract [9].

In addition, decreased levels of dietary and blood folate in autistic children has been identified as environmental contributors to the occurrence of ASD [45]. Accordingly, and to increase brain cells' antioxidant capacity in face of stress caused by MS, FA was used in this study. Supplementation of FA had similar results to previous studies regarding body weight [46], time of social contact and cognitive improvement [47]. Enhancement in behavioral status may be a result of improvement in NTs content which was related to oxidative and inflammatory status. Folates are linked to the synthesis of NTs through guanidine triphosphate needed for the production of tetrahydrobiopterin. The latter is a cofactor for monoamine synthesis [13].

Also, FA has acute antioxidant and free radical scavenging properties through heightening super oxide dismutase (SOD) and GPX levels in ischemic/reperfused rat [12]. Over and above, FA reversed the increase in serum level of IL-1β, NO and TNF-α, levels [16]. Mechanisms underlying the anti-inflammatory effects brought about by FA are still not yet fully interpreted [48]. Interestingly, it was proposed that, the intrinsic pathway of apoptosis is provoked by effects of ROS on mitochondria. This results in disruption of the equity between anti-apoptotic (bax) and pro-apoptotic (Bcl-2) proteins in favor of the latter leading to apoptosis [49]. Moreover, it was reported that FA decreased expression of bax and caspase-3 but, heightened expression of bcl-2 in cultured cortical neurons [50]. Consequently, the reduction in apoptosis by FA may be attributed to its antioxidant and anti-inflammatory activities.

Normalization of some parameters after dual administration of POM and FA indicate their better effect as protectors from autistic-like behavior than each one alone.

Conclusion

Our current results suggest that POM and/or FA protected against isolation induced autistic-like symptoms. They produced these effects through enhancement of redox state as well as repression of inflammation and apoptosis, which greatly reduced neurotransmitters loss in treated animals.

Disclosure

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