IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
ISSN-0973-1547 Volume **15** | Issue 1 | January, 2020 | 42-51
DOI: 10.15740/HAS/IJPS/15.1/42-51
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RESEARCH ARTICLE

Biochemical evidence for anti-autistic potential of *Asparagus racemosus*

■ **Priya Joon, Dinesh Dhingra and Milind Parle**

SUMMARY

Autism is a serious developmental disorder observed in early childhood that impairs the ability to communicate and interact socially. It is also characterized by a tendency to engage in repetitive behaviours, apathy and cognitive decline. *Asparagus racemosus* commonly known as *Shatavari* has been found to possess neuro-protective, nootropic, anti-depressant and anti-anxiety activities. In the light of above, a project was designed to study involvement of acetylcholine, catecholamines and oxidative stress in manifestation of autistic symptoms induced by valproic-acid in rat pups and their modulation by *Asparagus racemosus* (Shatavari). A single intraperitoneal injection of sodium valproate (500 mg/kg) was given on 13th day of gestation to pregnant Wistar female rats for inducing autism in rat pups. *Asparagus racemosus* root extract (100 and 200 mg/kg, p.o.) significantly reduced valproic acid- induced oxidative stress as indicated by decrease in plasma nitrite levels, increase in brain GSH levels and enhancement of catalase activity in brains of autistic rat pups. Furthermore, *Asparagus racemosus* (Shatavari) diminished acetylcholinesterase and monoamine oxidase-A enzyme activity in autistic pups. Shatavari restored valproic acid-induced biochemical deficits of rat pups in the present study. The present research findings, justify the status of Shatavari as a powerful medicinal herb for improving women's health. Autism spectrum disorder which has its origin in abnormal fetal development probably can be best treated by the use of this herb.

Key Words : Autism, Shatavari, Oxidative stress, AchE, MAO-A, Women-health

How to cite this article : Joon, Priya, Dhingra, Dinesh and Parle, Milind (2020). Biochemical evidence for anti-autistic potential of *Asparagus racemosus*. *Internat. J. Plant Sci.*, **15** (1): 42-51, **DOI: 10.15740/HAS/IJPS/15.1/42-51,** Copyright@ 2020: Hind Agri-Horticultural Society.

Article chronicle : Received : 14.11.2019; **Revised :** 09.12.2019; **Accepted :** 24.12.2019

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Autism is one of the neurological, developmental disorders due to abnormal wiring between different brain regions (Bhat *et al*., 2014). Although neurobiological mechanisms underlying Autism remain largely unknown, certain brain regions including limbic system, hippocampus, amygdala and cerebellum have been implicated in the clinical expression and pathophysiologic mechanism of ASD. Neuro-imaging and post-mortem studies mostly of adolescents have conferred evidence of structural abnormalities in above

regions of brain (Ecker *et al.*, 2015 and Ha *et al.*, 2015). Acetylcholine and catecholamines appear to play crucial role in memory dysfunction and depression. What differentiates autism from other disorders of development is the deviance, rather than the delay, in the developmental process. Autistic children usually show an admixture of bizarre behaviours and developmental deviance (Rutter and Schopler, 1987). An important hallmark of autism is that cognitive, sensory as well as motor development is retarded. The core symptoms of autism are centered aroundsocially inappropriate behaviour, poor expression, stereotypic behaviour, attention abnormalities and resistance to novel environment (Wagner *et al.*, 2006). Globally, autism is estimated to affect 24.8 million people (Geschwind and Matthew, 2015). In terms of gender, prevalence of autism in boysis four times higher as compared to girls (Newschaffer *et al.*, 2007). Prevalence rate of autism in children (1-10 years of age) in India was found to be 15/1000 and the highest prevalence rate was observed in the rural areas (Raina *et al.*, 2017).

Although no medicine has been recognized to treat this disorder, pharmacological treatments can be effective in reducing its signs, such as self-mutilation, aggression, repetitive and stereotyped behaviours, attention deficits, hyperactivity and sleeping disorders (Myers and Johnson, 2007). Currently, risperidone and aripiprazole are the only medications that have been reliably shown to be effective in ameliorating certain symptom clusters associated with ASD, such as disruptive behaviour and hyperactivity. Common side effects observed with use of risperidone include sedation, increased appetite, weight gain and elevated prolactin levels (Accordino *et al.*, 2016). Often such developmental disorders, which cannot be radically treated with allopathic medicines are managed with Ayurvedic system of medicine. In Ayurveda, *Asparagus racemosus* (Shatavari) is known as the "Queen of herbs". This amazing herb is considered not only as a general tonic but also as a female reproductive tonic. *Asparagus racemosus* is a well known Ayurvedic rasayana, which prevents ageing, increases longevity, imparts immunity, improves mental function and addsvitality to the body (Alok *et al.*, 2013).

In the light of above, a project was designed to study involvement of acetylcholine, catecholamines and oxidative stress in manifestation of autistic symptoms and their modulation by Shatavari.

MATERIAL AND METHODS

Experimental animals:

This study was carried out on pregnant female ratspurchased from Disease Free Small Animal House of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India). These pregnant rats were housed individually in separate cages (polypropylene cage size: 43×27×15 cm) for acclimatization to laboratory conditions with alternating light and dark cycle of 12 h each. The animals had free access to food and water. The experimental protocol was approved by In- stitutional Animals Ethics Committee. Animal care was taken as per the guidelines of CPCSEA, Govt. of India (Registration number 436/PO/ReBi/S/01/ CPCSEA).

Plant material:

The dried roots of *Asparagus racemosus* commonly known as Shatavari, were purchased from local market of Rohtak (Haryana) and authenticated by ICAR-National Bureau of Plant Genetic Resources, Division of Plant Exploration and Germplasm Collection National Herbarium of Cultivated Plants (NHCP), New Delhi (NHCP/NBPGR/2017-18) as *Asparagus racemosus* Willd., (family- Liliaceae/Asparagaceae.)

Extraction:

The extract of *A. racemosus* was prepared as per the method described earlier (Dhingra and Goyal, 2008). The dried roots were grounded to coarse powder. About 400 g of powdered drug was extracted with ethanol (95% v/v) using Soxhlet apparatus at 78°C till siphoning solution became colourless. The extract was concentrated by rotary vacuum evaporator and dried by using water bath. The concentrated extract was dark brown in colour and percentage yield was 13 per cent (w/w). The extract was stored in air tight container and kept in a refrigerator for further studies.

Selection of doses:

On the basis of literature (Dhingra and Kumar, 2012) and pilot study, the doses of *A. racemosus* extract were selected as 50, 100 and 200 mg/kg, p.o.

Experimental design:

Induction of autism:

For induction of autism disorder, a single intraperitoneal injection of sodium valproate (500 mg/

kg) was given on 13thday of gestation. Both, sodium valproate-treated and control female rats were housed individually in separate cages and allowed to raise their own litters under laboratory conditions. On post natal day (PND) 20, the offsprings were weaned out for carrying out further experiments. In pups, autism disorder was confirmed by applying early post natal developmental tests (Schneider and Przewlocki, 2005). Development of autism in pups was reflected by evaluation of litter size, eye opening day, body weight and olfactory discrimination, which was then compared with normal control rat pups.

Early post natal development (PND) tests:

Decrease in body weight (measured on PND 7,14, 21, 28 and 35), delayed eye opening (observed once daily) and impaired olfactory discrimination (observed on PND 9) are the tests that manifested autistic symptoms in early developmental stage (Schneider and Przewlocki, 2005).

Olfactory discrimination:

This test reflects a nest-seeking response mediated by the olfactory system (Gregory and Pfaff, 1971). Olfactory discrimination test was performed on PND 9 as per procedure followed by Schneider and Przewlocki, 2005 with slight modification. The apparatus consisted of a polycarbonate cage ($27\times21\times14cm^3$). A line was drawn on each end of the cage at a distance of approximately 4 cm. One end of the apparatus was filled with fresh bedding, while the other end was filled with home cage bedding. Three days old bedding was considered as home- bedding. A 3 cm^2 area demarcated the centre of the cage. Each pup was placed in the centrally demarcated area and latency timetaken by the pup to enter the home -bedding side by crossing the designed line with the front paws and head was recorded. Central placement of the pup was balanced by alternating the pup facing to or away from the experimenter.

Experimental protocol:

On PND 20, pups were weaned out and divided in following groups having six pups in each group. Group I: Control (vehicle treated) received distilled water per oral; Group II: VPA (500 mg/kg, i.p.); Groups III: received VPA (500 mg/kg, i.p.) and AR (50 mg/kg p.o); Group IV: received VPA (500 mg/kg, i.p.) and AR (100 mg/kg p.o); Group V: received VPA (500 mg/kg, i.p.) and AR (200 mg/kg p.o); Group VI: received VPA (500 mg/kg, i.p.) and Fluoxetine (10 mg/kg, i.p); Group VII: received VPA (500 mg/kg, i.p.) and Donepezil (0.75 mg/kg, i.p); Group VIII - Group XII: received same treatment as Groups I-V.

These Pups received treatment (Test drug and Standard drugs) individually as per their above assigned groups from 21st to 35th postnatal days. Biochemical estimations were performed in plasma and brains of pups. In animals of groupsI-VI, plasma nitrite levels, brain reduced glutathione level, brain catalase and brain MAO-A levels were estimated. In animals of groups VII to XII, brain acetylcholinesterase activity was estimated.

Biochemical estimation in plasma:

Rat pups of groups I- VI were sacrificed by cervical dislocation under light anesthesia with chloroform. This was followed by withdrawal of blood sample (about 1 ml) from carotid artery bleeding. Blood samples were centrifuged at 2400 rpm for 10 min using refrigerated centrifuge to separate plasma, which was then used for estimation of nitrite levels.

Estimation of plasma nitrite level:

Plasma nitrite was measured by using the method of Green *et al.* (1982). A mixture of 1%w/v sulphanilamide in 5% aqueous solution of m-phosphoric acid and 0.1%w/v N-(1-Naphthyl) ethylene di-amine hydrochloride was prepared. This mixture was refrigerated at 0°C for 60 min. 0.5 ml plasma was mixed with 0.5 ml of the above mixture and kept in dark for 10 min at room temperature. The absorbance was read at 546 nm using UV-visible spectrophotometer.

Biochemical estimations in brain samples:

The brains from animals of groups I- VI were isolated after collecting blood samples. These brain samples were washed with cold 0.25M sucrose-0.1M Tris-0.02M EDTA buffer (pH 7.4) and weighed. The buffer washed brain sample was homogenized in 9 volumes of cold 0.25M sucrose-0.1M Tris-0.02M EDTA buffer (pH 7.4) and centrifuged twice at 2500 rpm for 10 min at 4°C in cooling centrifuge. The supernatant fluid was then centrifuged at 4°C in cooling centrifuge at 12000 rpm for 20 min and then separated into two parts (A and B):

Part A:

The precipitate mitochondrial fractionwas used for estimation of MAO- A activity.

Part B:

The remaining supernatant fluid was used to assay reduced glutathione and catalase levels.

Estimation of MAO- A activity:

The MAO-A activity was assessed spectrophotometrically (Schurr and Livne, 1976) at wavelength of 280 nm for 5 min against the blank containing sodium phosphate buffer and 5-HT.

Estimation of reduced glutathione activity:

Reduced glutathione was assayed by the method of Jollow *et al.* (1974). 1.0 ml of supernatant fluid (10%) was precipitated with 1.0 ml of sulfosalicylic acid (4%). The samples were kept at 4°C for at least 1h and then subjected to centrifugation at 1200 rpm (15 min) at 4°C. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1M, pH 7.4) and 0.2 ml 5,5 dithiobis-(2-nitro benzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm using UV-visible spectrophotometer. Reduced glutathione levels were calculated using molar extinction co-efficient of 1.36×10^4 M^{-1} cm^{-1} and expressed as micromole per milligram protein.

Estimation of catalase activity:

Catalase activity was assayed by the method of Claiborne (1985). The assay mixture consisted of 1.95 ml phosphate buffer (0.05M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml post-mitochondrial supernatant fluid (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm using UV-Visible Spectrophotometer. Catalase activity was quantified using the milli-molar extinction co-efficient of H_2O_2 (0.07 mM) and expressed as micromoles of H_2O_2 decomposed per minute per milligram protein.

Estimation of total protein concentration:

Total protein concentration was estimated in brain homogenate of pups by using a total protein kit (Liquixx Total Protein, ERBA diagnostics Mnnheim GmbH), using semi-automatic autoanalyzer (Chem 5 plus-V2 semi-autoanalyzer; ERBA Mannheim, Germany).

Estimation of AchE activity:

Animals of groups VII toXII were sacrificed using cervical dislocation and immediately brain was removed, washed, weighed and homogenized in phosphate buffer (pH 8, 0.1M) and then centrifuged at 3000 rpm for 10 min at 4°C in a cooling centrifuge. The supernatant so obtained was used for the estimation of AchE activity by following the method of Ellman *et al.* (1961), with slight modifications. The reaction mixture consisted of 0.4 ml separated supernatant, 0.1ml DTNB [5,5-dithio-bis-(2-nitrobenzoic acid)] and 2.6 ml of 0.01M sodium phosphate buffer (pH 8) and its absorbance was observed on UV–Visible Spectrophotometer at 412nm. Further, 0.02ml acetyl-thiocholine iodide solution was added and change in absorbance was recorded for 3 min at intervals of 1 min at 412 nm. The change in absorbance per minute was determined and enzyme activity was calculated.

Statistical analysis:

All the results were expressed as Mean ± SEM. Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test.

RESULTS AND DISCUSSION

VPA (500mg/kg) administration to pregnant female rats significantly affected normal development of fetus. Parameters in newborn rat pups, such as body weight, eye opening day, olfactory impulses, and variable changes in biochemical parameters were observed. VPA treated rat pups were found to be autistic, when judged on these parameters.

Effect on litter size:

Intraperitoneal administration of valproic acid (500 mg/kg) on 13th day of gestation significantly ($p<0.0001$) reduced litter size in valproate treated (5.06 ± 0.20*) pregnant female rats as compared to control group (10.25 ± 0.46).

Early post natal development (PND) tests:

Effect on eye opening:

Valproic acid (500 mg/kg, i.p.) significantly ($p<0.0001$) delayed opening of eyes in valproate treated (20.59 ± 0.07*) rat pups as compared to control group (15.00 ± 0.09).

Effect on olfactory discrimination:

Olfactory discrimination of home bedding odor was investigated in valproate treated rat pups. Autistic rat pups had difficulty in identifying home bedding. Valproic

acid (500 mg/kg, i.p.) significantly ($p<0.0001$) increased the mean latency time to reach home bedding (35.33 ± 1.53* sec) on PND 9 as compared to control group (7.91 ± 0.80 sec).

Effect on body weight of rat pups:

Valproic acid (500 mg/kg, i.p.) significantly ($p<0.0001$) reduced body weight of rat pups in chronological fashion, when measured on post natal days 7, 14, 21, 28 and 35 as compared to control group as shown in Fig.1.

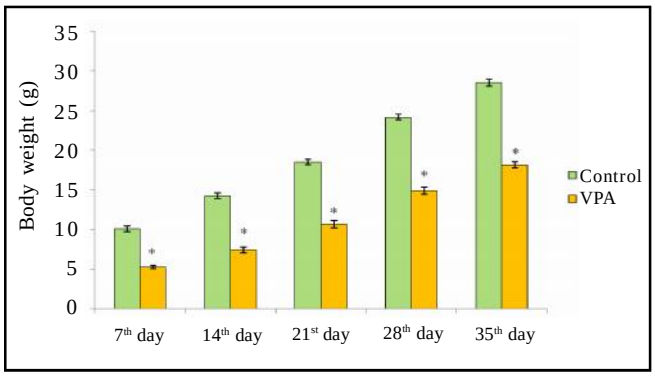

Fig. 1: Effect of valproic acid on body weight of rat pups

n=12 in each group
Data are expressed as Mean ± SEM and analyzed by unpaired t-test.
VPA stands for Valproic acid.
*<0.0001 (7th day), *p<0.0001 (14th day), *p<0.0001 (21st day), *p<0.0001 (28th day) and mp<0.0001 (35th day) as compared to control group.

Effect of ethanolic extract of *Asparagus racemosus* roots on various biochemical parameters:

Effect of Asparagus racemosus on plasma nitrite levels:

Administration of Valproic acid (500 mg/kg, *i.p.*) to pregnant female rats on 13th day of gestation, significantly ($p<0.001$) increased total plasma nitrite levels of their offsprings as compared to normal pups (control group). Treatment with ethanolic extract of *Asparagus racemosus* 100 mg/kg ($p<0.01$) and 200 mg/kg ($p<0.001$), *p.o.* for 14 consecutive days significantly reversed valproic acid-induced increase in plasma nitrite levels of autistic pups. Treatment with standard drug fluoxetine (10 mg/kg, *i.p.*) also decreased plasma nitrite levels of autistic pups significantly ($p<0.001$) like *Asparagus racemosus*. The lowest dose (50 mg/kg, *p.o.*) of extract did not ($p>0.05$) affect plasma nitrite levels of autistic pups (Fig. 2).

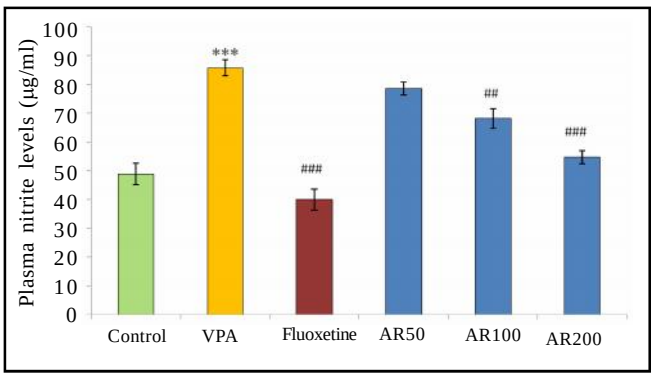

Fig. 2: Effect of ethanolic extract of *Asparagus racemosus* on plasma nitrite levels of rat pups

n= 6 in each group
Data are expressed as Mean ± SEM and analyzed by One- Way ANOVA followed by Tukey's test.
VPA stands for Valproic acid and *AR50*, *AR100* and *AR200* stand for *Asparagus racemosus* 50, 100 and 200 mg/kg, p.o. respectively.
***p<0.001 as compared to vehicle treated control
##p<0.01 and ###p< 0.001 as compared to valproic acid treated

Effect of Asparagus racemosus on reduced Glutathione (GSH) levels:

Valproic acid (500 mg/kg, *i.p.*) administration to pregnant female rats on 13th day of gestation, significantly ($p<0.001$) reduced the GSH levels in brains of their pups as compared to control group. Treatment with ethanolic extract of *Asparagus racemosus* (100 mg/kg and 200 mg/kg, *p.o.*) for 14 consecutive days significantly ($p<0.001$) reversed valproic acid-induced reduction in brain GSH levels of autistic pups. Treatment of autistic

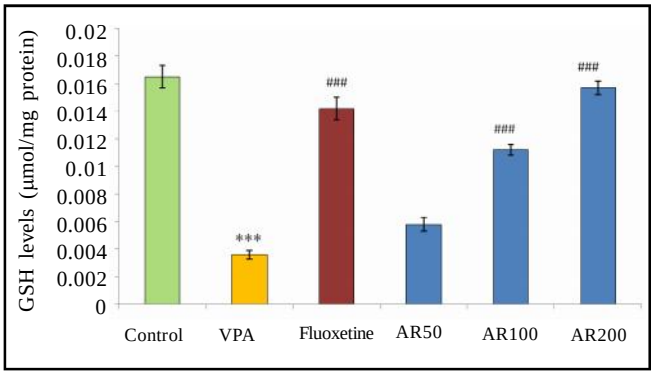

Fig. 3: Effect of ethanolic extract of *Asparagus racemosus* on brain GSH levels of rat pups

n= 6 in each group
Data are expressed as Mean ± SEM and analyzed by One- Way ANOVA followed by Tukey's test.
VPA stands for Valproic acid and *AR50*, *AR100* and *AR200* stand for *Asparagus racemosus* 50, 100 and 200 mg/kg, p.o. respectively.
***<0.001 as compared to vehicle treated control.
###p< 0.001 as compared to valproic acid treated group.

pups with standard drug fluoxetine (10 mg/kg, *i.p.*) increased the brain GSH levels (Fig. 3) of autistic pups significantly ($p<0.001$).

Effect of Asparagus racemosus on brain Catalase activity:

Administration of Valproic acid (500 mg/kg, *i.p.*) to pregnant female rats on 13th day of gestation, significantly ($p<0.001$) reduced catalase enzyme activity in brains of their offsprings as compared to control group. Treatment with ethanolic extract of *Asparagus racemosus* (100 mg/kg and 200 mg/kg, *p.o.*) for 14 consecutive days significantly ($p<0.001$) enhanced brain catalase activity of autistic pups. Treatment with standard drug fluoxetine (10 mg/kg, *i.p.*) also enhanced the brain catalase activity of autistic pups significantly ($p<0.001$). The lowest dose (50 mg/kg, *p.o.*) of extract did not ($p>0.05$) affect brain catalase activity of rat pups (Fig. 4).

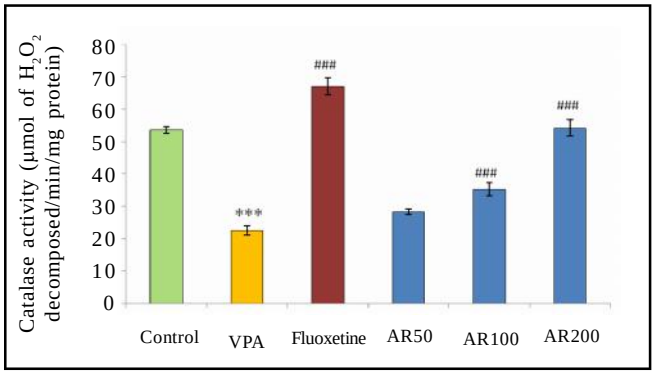

Fig.4: Effect of ethanolic extract of *Asparagus racemosus* on brain catalase activity of rat pups

n= 6 in each group
Data are expressed as Mean ± SEM and analyzed by One- Way ANOVA followed by Tukey's test.
VPA stands for Valproic acid and *AR50, AR100* and *AR200* stand for *Asparagus racemosus* 50, 100 and 200 mg/kg, p.o. respectively.
*** <0.001 as compared to vehicle treated control.
###p< 0.001 as compared to valproic acid treated group.

Effect of Asparagus racemosus on brain MAO-A activity:

Offsprings of pregnant female rats administered with Valproic acid (500 mg/kg, *i.p.*) on 13th day of gestation showed significant ($p<0.001$) increase in Monoamine oxidase enzyme-A (MAO-A) activity in their brains as compared with pups of control group. Administration of ethanolic extract of *Asparagus racemosus* 100 mg/kg ($p<0.01$) and 200 mg/kg ($p<0.001$), *p.o.* for 14 consecutive days significantly reversed valproic acid-induced increase in brain MAO-A activity of autistic pups. The lowest dose (50 mg/kg, *p.o.*) of extract did not ($p>0.05$) affect the brain MAO-A activity of valproic acid-induced autistic pups (Fig. 5).

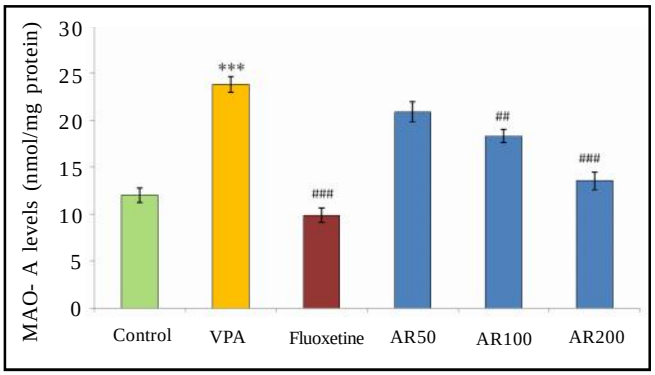

Fig.5: Effect of ethanolic extract of *Asparagus racemosus* on brain MAO-A activity of rat pups

n= 6 in each group
Data are expressed as Mean ± SEM and analyzed by One- Way ANOVA followed by Tukey's test.
VPA stands for Valproic acid and *AR50, AR100* and *AR200* stand for *Asparagus racemosus* 50, 100 and 200 mg/kg, p.o. respectively.
*** <0.001 as compared to vehicle treated control.
p< 0.01 and ###p< 0.001 as compared to valproic acid treated group.

Effect of Asparagus racemosus on brain Acetylcholinesterase activity:

Administration of Valproic acid (500 mg/kg, *i.p.*) to pregnant female rats on 13th day of gestation significantly ($p<0.001$) increased acetylcholinesterase (AchE) enzyme activity in brains of their offsprings as compared

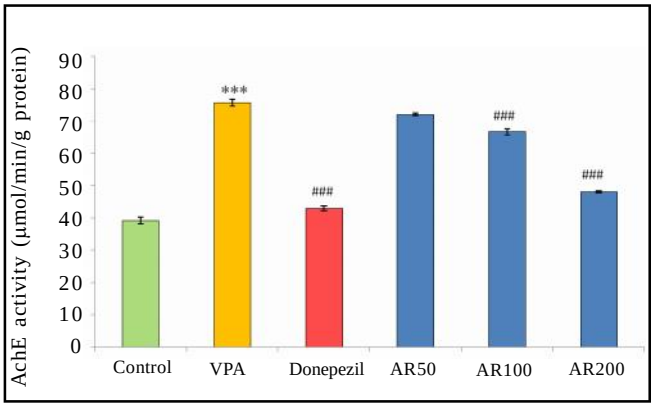

Fig.6: Effect of ethanolic extract of *Asparagus racemosus* on brain AchE activity of rat pups

n= 6 in each group
Data are expressed as Mean ± SEM and analyzed by One- Way ANOVA followed by Tukey's test.
VPA stands for Valproic acid and *AR50, AR100* and *AR200* stand for *Asparagus racemosus* 50, 100 and 200 mg/kg, p.o. respectively.
***<0.001 as compared to vehicle treated control.
###p< 0.001 as compared to valproic acid treated group.

to control group. Treatment with ethanolic extract of *Asparagus racemosus* (100 mg/kg and 200 mg/kg, p.o.) significantly ($p<0.001$) reversed valproic acid-induced increase in brain AchE activity of autistic pups. Treatment with standard drug donepezil (0.75 mg/kg, *i.p.*) also decreased the AchE activity in brain of autistic pups like *Asparagus racemosus*. The lowest dose (50 mg/kg, *p.o.*) of extract did not ($p>0.05$) affect the brain AchE activity of valproic acid-induced autistic pups (Fig. 6).

Toxicity studies:

Toxicity studies were carried out as per OECD guidelines in order to ascertain safety of the plant selected. *Asparagus racemosus* was found to be non-toxic even at the dose range of 500- 2000mg/kg, *p.o.*

Autism spectrum disorder (ASD) is a neuropsychiatric disorder occurring in infants of 1-4 years of age, characterized by mental retardation, defective social interaction, apathy and bizarre behaviour. Recently, ASD has been redefined in The Diagnosticand Statistical Manual of Mental Disorders (DSM)-5 of American Psychiatric Association. Previous three criteria of ASD, qualitative impairment in social interaction, in communication and restricted repetitive and stereotyped patterns of behaviour, interests and activities, have been reconstructed into two domains, persistent deficits in social communication and social interaction, restricted, repetitive patterns of behaviour, interests, or activities (Ha *et al.*, 2015) Understanding of Autism Spectrum Disorder (ASD) has constantly expanded, though etiology and pathophysiology of this disorder are still a matter of speculation. However, ASD continues to be defined only by behavioural symptoms in the absence of reliable biomarkers. Therefore, we were interested to study the influence of certain biomarkers such as plasma nitrite levels, reduced glutathione levels, catalase levels, MAO-A and AchE levels in the development of Autism. Nevertheless, we believe that biochemical alterations taking place during pathogenesis of autism could open a new dimension in understanding this complex disorder.

In utero-exposure of rodents to valproic acid (VPA) has been proposed to induce behavioural characteristics similar to those observed in ASD. Sodium valproate-induced autism in rodents such as rats and mice is a robust animal model of autism to evaluate the effects of medicines potentially useful in autism (Sandhya *et al.*, 2012 and Wagner *et al.*, 2006). Pregnant rats exposed to sodium valproate on 13th day of gestation, (the time of neural tube closure) (Alsdorf and Wyszynski, 2005) gave birth to abnormal offsprings, which exhibited bizarre behaviour and several anatomical abnormalities in the brain stem and cerebellum resembling to those found in autopsy and brain imaging studies of autistic patients (Kim *et al.*, 2011). In the present study, administration of VPA to pregnant female rats on 13thday of gestation; produced abnormal pups as manifested by unusually low body weight at birth, impaired olfactory discrimination and delayed eye opening. This observation is in agreement with literature reports, which indicate production of autistic pups by VPA. Pre-natal exposure to environmental pro-oxidants such as valproic acid triggers the formation of reactive oxygen species, which interfere with the neurodevelopmental process and cause neural damage, resulting in abnormal behaviour (Chauhan and Chauhan, 2006). *Asparagus racemosus* is commonly known as "Shatavari, meaning "a lady possessing immense feminine powers, who can please hundred husbands". Two main texts of Ayurvedic medicines, *viz.*, Charak Samhita and Ashtang Hridyam, make a mention of *Asparagus racemosus* as a wonderful herb for strengthening women's health (Thorne, 2000). In modern Ayurvedic practices the roots of this plant are considered to be possessing anti-spasmodic, appetizer, aphrodisiac, galactogogue, anti-diarhoeal, anti-dysentiric, laxative, anti-cancer, anti-inflammatory, anti-tubercular, neuro-protective, nootropic, anti-depressant, anti-anxiety anti-epileptic and nephro-protective properties (Garde and Sarth, 1970 and Hasan *et al.*, 2016). Therefore, this project focussed on biochemical evidence for anti-autistic potential of Shatavari in rat pups.

Autism appears to be the net result of a combination of genetic, environmental and immunological offenders precipitated by oxidative stress. Oxidative stress in autism has been studied at the membrane level. Furthermore, the concentrations of the products of lipid peroxidation, reduced glutathione and endogenous antioxidants involved in the defense system have been investigated. Lipid peroxidation markers are elevated in autism indicating increased oxidative stress. Levels of major antioxidant serum proteins namely transferrin (iron-binding protein) and ceruloplasmin (copper- binding protein) are decreased in children with autism. There appears to be a positive correlation between reduced levels of these protective proteins and loss of expression skills in autistic children. Several studies have suggested

alterations in the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase in the pathogenesis of autism (Chauhan and Chauhan, 2006). In the present study, enhanced levels of plasma nitrite and diminished reduced glutathione (GSH) and catalase levels confirm involvement of oxidative stress in VPA induced autism. On the other hand, treatment with ethanolic extract of *Asparagus racemosus* roots at higher doses (100 mg/kg and 200 mg/kg, *p.o.*) for 14 consecutive days significantly reversed valproic acid-induced enhanced plasma nitrite levels, as well as diminished brain GSH and catalase levels of autistic pups.The findings of present study revealed anti- autistic potential of *A. racemosus* owing to its anti-oxidant effect observed in the present study. Furthermore, neuro-inflammation, excito-toxicity and immune dysfunction has been suggested to occur in autistic children. *A. racemosus* possess potent neuro-protective property (Uddin *et al.*, 2016) which might have contributed favourably in reversing autistic symptoms of rat pups. *A. racemosus* significantly restored the altered oxidative stress markers in autistic pups due to its antioxidant property (Kamat *et al.*, 2000).

In the present study, valproic acid exposure to pregnant female rats on 13th day of gestation resulted in birth of autistic pups with increased Monoamine oxidase enzyme-A (MAO-A) activity in brain. This enhanced activity of MAO-Ain brains of autistic pups was reversed by treatment with ethanolic extract of *Asparagus racemosus* roots at higher doses (100 mg/kg and 200 mg/kg, *p.o.*) in the present study. These observations are in line with literature reports. The monoamine system is altered in patients with autism (Gottfried *et al.*, 2013). Monoamine oxidases (MAOs) catalyze the metabolism of monoamine neurotransmitters, such as serotonin, dopamine and norepinephrine, which are key regulators for normal brain function. Mono-aminergic neurotransmitters play a critical role in the modulation of mood, emotions, motor activity, behaviour and perception. Decreased activity of MAOs lead to increased levels of these mono-aminergic neurotransmitters. Several studies suggested impaired MAO-A activity in the brains of children suffering from autism. Serotonin has been shown to enjoy a major role in mood swings of autism (Gu *et al.*, 2017). It's interesting to note that serotonin levels were found to be abnormal in autistic subjects (Muller *et al.*, 2016). Wu and Shih (2011) reported that VPA activates monoamine oxidase-A (MAO- A) catalytic activity.

It has been reported that around 70 per cent of people suffering with autism exhibit cognitive deficits. Cholinergic system influenceslearning index and memory process in healthy (Kaura and Parle, 2016) as well as autistic children (Baradaran *et al.*, 2012). Furthermore, there is an evidence of dysregulated cholinergic system in the brains of ASD patients. Acetylcholine (ACh) is the main neurotransmitter implicated in various neurological processes such as plasticity, cognition, memory and release of other neurotransmitters especially in the central nervous system (Sarter and Bruno, 2004; Barnes *et al.*, 2000; Cutuli *et al.*, 2009; Sarter and Paolone, 2011 and Picciotto *et al.*, 2012). Acetylcholine is synthesized from precursors acetyl CoA and choline in the presence of choline acetyl transferase (ChAT) and is degraded by acetylcholinesterase (AChE). The dysfunction of these enzymes and cholinergic receptors cause many neurological disorders. Studies indicate that prenatal exposure of valproic acid (VPA) to fetus caused impairment of cholinergic neuronal development in fetus, most notably by up-regulation of acetylcholinesterase (AChE) enzyme (Kim *et al.*, 2014). This may be probably responsible for defective learning and memory processes in autistic rat pups. In the present study, valproic acidexposure to pregnant female rats on 13th day of gestation resulted in birth of autistic pups with high levels of acetylcholinesterase (AchE) enzyme activity in brain. Whereas, treatment with ethanolic extract of *Asparagus racemosus* roots at higher doses (100 mg/kg and 200 mg/kg, p.o.) for 14 consecutive days to autistic rat pups reduced brain AchE activity, thereby indicating nootropic effect of *A. racemosus* extract. This finding is in concurrence with the reports of Ojha *et al.* (2010), who demonstrated that *A. racemosus* extract improved memory of amnesic rats and Lalert *et al.* (2013) who showed that *A. racemosus* root extract is capable of improving memory function in ovariectomized rats. It may be noteworthy that spatial memory of autistic rat pups was improved by administration of ethanolic extract of *A. racemosus* roots.

Conclusion:

In the light of above literature reports and present research findings, Shatavari appears to justify its status as a powerful medicinal herb for improving women's health. Autism spectrum disorder, which has its origin in abnormal fetal development probably can be best treated

by the use of this herb.

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