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RESEARCH **P**APER

Shatavari: A nature's gift for autism

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Autism is a serious developmental disorder seen 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. Early prenatal or post natal exposure to neuro-toxicants such as valproic acid (VPA), thalidomide and ethanol induce behavioral alterations similar to autistic symptoms. *Asparagus racemosus* commonly known as *Shatavari* has been found to possess neuro-protective, nootropic, antidepressant, and antianxiety activities. Therefore, the present study was undertaken to investigate the effect of the root extract of *Asparagus racemosus* on valproic-acid induced autism in rat pups. 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.) administered alone significantly reversed valproic acid induced hyperlocomotion, anxiety, memory impairment, increased sensitivity to pain and depression-like behaviour in pups. Shatavari restored valproic acid-induced behavioural deficits of rat pups in the present study probably via its anti-anxiety, anti-depressant, nootropic, anti-nociceptive and neuro-protective properties.

Key words : Autism, *Asparagus racemosus*, Sodium valproate, Rat pups

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INTRODUCTION

Autism Spectrum Disorder (ASD) is a complex neurological pervasive developmental disability, which occurs during 1-3 yrs of age in infants. It is a neuropsychiatric syndrome characterized by mental retardation, defective social interaction, obsessions, impaired communication, apathy, and unusual behaviour. ASD patients frequently have several complications such as epilepsy, depression, anxiety and language impairment (Kas *et al.*, 2014 and Jeste and Tuchman, 2015). Children suffering from Autism have intellectual disability, exhibiting very low IQ of less than 70 (Carper and Courchesne, 2005). An estimated 62 million cases of ASD have been reported worldwide. Its prevalence is four times more common in boys than in girls (Newschaffer *et al.*, 2007). Autism is reported to occur in all racial and ethnic groups, particularly in developed countries, ASD prevalence has been estimated to be about 1 per cent across all ages. In India, It has been estimated that more than 2 million people might be affected with ASD (Krishnamurthy, 2008). Autism is one of the psychiatric and heterogeneous developmental disorders due to abnormal wiring between different brain regions (Bhat *et al.*, 2014). Autism can be caused by both genetic and non-genetic factors (Muhle *et al.*, 2004) inclusive of environmental neuro-toxicants (Wagner *et al.*, 2006). Pathophysiological changes occurring in autism include dysregulated inflammatory cascades, neuronal degeneration, microglial cell activation, and oxidative stress in the brain during fetal development in neurological disorders (Marshall *et al.*, 2008). The onset of ASD

reportedly involves numerous factors such as genetic variation, epigenetic anomalies, environmental factors, dysfunction of the GABAergic system in the brain (Oblak *et al.*, 2009; Oblak *et al*., 2010; Coghlan *et al.*, 2012 and Han *et al.*, 2014). Since, ASD is an incurable disorder; quality of life of children suffering from autism as well as their families is highly compromised. Clinical treatment of autism aims at improving the quality of life of affected children and reduce the family distress Although, no medicine is available to cure this disorder, pharmacological treatments can be effective in reducing its clinical signs, such as depression, aggression, repetitive and stereotyped behaviors, lack of concentration, hyperactivity, and sleeping disorders (Myers and Johnson, 2007). Modern medicines have been reported to evoke undesirable but inevitable ill effects having poor patient compliance. Therefore, herbal medicines offer huge scope in the search of an effective remedy for brain disorders. India has a huge reservoir of medicinal plants effective in the management of brain related disorders. Shatavari is a species of Asparagus plant that has been used for many centuries in Indian Ayurvedic medicine. Shatavari also known as 'Shatavar' or *Asparagus racemosus* is found to promote fertility in addition to having a range of health benefits. Shatavari specifically is useful as an anti inflammatory agent, immune booster, a diuretic and cough reliever. Furthermore, Shatavari is a powerful galactogogue (Alok *et al.*, 2013). *Asparagus racemosus* roots have also been reported to possess anti-convulsant (Shastry *et al.*, 2015) and neuroprotective effect (Uddin *et al.*, 2016).

Therefore, this study was undertaken to investigate the possible beneficial effect of *Asparagus racemosus* in ameliorating autistic symptoms of rat pups.

RESEARCH METHODOLOGY

Experimental animals:

This study was carried out on pregnant female rats purchased from Disease Free Small Animal House of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India) on 5th day of gestation. 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 Institutional Animals Ethics Committee. Animal care was taken as per the guidelines of CPCSEA, Govt. of India (Registration No. 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 colorless. 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 12.98% (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, the doses of *A. racemosus* extract (50, 100 and 200 mg/kg, p.o.), (Dhingra and Kumar, 2007) were selected.

Vehicle:

Asparagus racemosus extract; fluoxetine, diazepam, diclofenac sodium and donepezil were dissolved separately in distilled water just before administration.

Experimental design:

Induction of Autism:

For induction of autism disorder, a single intraperitoneal injection of sodium valproate (500 mg/kg) was given on 13th day 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 time taken 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 Diazepam (0.5 mg/kg, i.p); Group VIII: received VPA (500 mg/kg, i.p.) and Diclofenac sodium (10 mg/kg, i.p); Group IX: received VPA (500 mg/kg, i.p.) and Donepezil (0.75 mg/kg, i.p); Group X- Group XIV: 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. These offsprings were subjected to various behavioral tests during last two days of treatment (34th and 35th day). Behavioral tests performed in Rat pups of group I to VIII were- Tail Immersion Test (Vogel, 2008) for nociception (on 34th day); Tail Suspension Test (Steru *et al.*, 1985)- for depression (on 34th day); Elevated Zero Maze Test (Kulkarni, 2008)- for anxiety (on 35th day); Actophotometer Test (Kumar *et al.*, 2011 and Chhillar and Dhingra, 2013)- for locomotor activity (on 35thday). While in rat pups of group IX to XIV, the behavioral tests performed were specific for learning and memory including Morris Water Maze test (34thday to 38thday).

In animals of Groups I-VIII, there was an interval of 1.5 hours between two different behavioral test exposures. The experiments were performed between 9 AM to 5 PM.

Morris water maze test:

The retention and acquisition of a spatial navigation task were examined by using a Morris water maze (Dhingra and Kumar, 2012 and Morris, 1984). Rat pups were trained to find a submerged platform (10×10 cm^2) in a cylindrical pool (60 cm in diameter and 25 cm in height) of opaque water (using titanium oxide), with 1cm below the surface of the water in the middle of the target quadrant (north Q1). The position of the platform was kept unaltered throughout the training session. The water temperature maintained was 28 ±°C and measuring 20 cm deep. The time spent by rat pups in target quadrant was recorded and tabulated. This activity was measure for four days from 34th to 38th PND. Donepezil (0.75 mg/kg, i.p.) treated rat pups were taken as standard control group for comparative evaluation of AR extract on memory and recognition ability of VPA induced ASD in rat pups.

Acquisition test (Learning):

All the rat pups were allowed to swim over four consecutive days and consisting of 4 swimming trials per day, each at an interval of 30 min approximately. Each rat pup was subjected to training trials for 4 consecutive days, the starting position was changed with each

exposure as mentioned below and target quadrant (Q1) remained constant throughout the training period:

Day 1 Q1 Q2 Q3 Q4
Day 2 Q2 Q3 Q4 Q1
Day 3 Q3 Q4 Q1 Q2
Day 4 Q4 Q1 Q2 Q3

For each trial, the rat pup was placed at the edge of the pool in the centre of the appropriate quadrant, facing the wall of water maze; and latency to find the platform was recorded. Cut off time for finding the platform was kept 60 sec. If the animal finds the platform before the 60 sec cut-off, it was allowed to stay on the platform for 5 seconds and then return it to its home cage. If the animal could not find the submerged platform in 60 sec, then the animal was gently placed on it and allowed to stay there for the next 15sec before returning it to home cage. Escape latency time to locate the hidden platform in water maze was noted as an index of acquisition or learning.

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.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads:

Effect of ethanolic extract of *Asparagus racemosus* on various behavioral models:

Effect of Asparagus racemosus on tail withdrawal reflex of rat pups using Tail Immersion Test:

Valproic acid (500 mg/kg, *i.p.*) significantly ($p<0.05$) induced hyperalgesia in animals as indicated by decreased time of tail withdrawal reflex. Treatment with ethanolic extract of *Asparagus racemosus* 100 mg/kg ($p<0.01$) and 200 mg/kg ($p<0.001$) *p.o.* per se for 14 consecutive days significantly reversed valproic acid- induced hyperalgesia in rat pups. Treatment with standard drug diclofenac sodium (10mg/kg, *i.p.*) also reversed valproic acid induced hyper-algesic effect significantly ($p<0.001$). The lowest dose of the extract (50 mg/kg, *p.o.*) did not significantly ($p>0.05$) affect tail withdrawal reflex time of pups (Table 1).

Influence of *Asparagus racemosus* on anxious behavior of rat pups using Elevated Zero Maze:

Valproic acid treatment (500 mg/kg, *i.p.*) significantly ($p<0.001$) decreased number of entries and time spent in open arms as compared to control. Treatment with ethanolic extract of *Asparagus racemosus* 100 mg/kg ($p<0.01$) and 200 mg/kg, ($p<0.001$) *p.o.* per se for 14 consecutive days significantly antagonized valproic acid induced anxious behavior as indicated by increase in number of entries and time spent in open arms. Treatment with standard drug diazepam (2 mg/kg, *i.p.*) also increased the number of entries and time spent in open arms. The lowest dose (50 mg/kg, *p.o.*) of the extract did not significantly ($p>0.05$) affect the anxious behavior of rat pups (Table 2).

Effect of *Asparagus racemosus* on locomotor activity of rat pups using actophotometer:

Valproic acid (500 mg/kg, *i.p.*) significantly ($p<0.001$)

Table 1 : Effect of ethanolic extract of *Asparagus racemosus* and diclofenac sodium (standard drug) on tail withdrawal reflex (nociception) of rat pups using Tail Immersion Test

Sr. No.	Treatment	Dose per kilogram	Tail Withdrawal Reflex (sec)
1.	Control (Distilled Water)	10 ml, p.o.	2.66 ± 0.21
2.	VPA	500 mg, i.p.	1.16 ± 0.16*
3.	VPA + Diclofenac sodium	10 mg, i.p.	7.16 ± 0.30$^{###}$
4.	VPA + *AR*	50 mg, p.o.	2.33 ± 0.49
5.	VPA + *AR*	100 mg, p.o.	3.50 ± 0.50$^{##}$
6.	VPA + *AR*	200 mg, p.o.	4.83 ± 0.47$^{###}$

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 *AR* stands for *Asparagus racemosus.*
$F(5, 30) = 30.57$, $p< 0.0001$.
*$p< 0.05$ as compared to vehicle treated control.
$^{##}p< 0.01$, $^{###}p< 0.001$ as compared to valproic acid treated group.

increased the locomotor activity as compared to control group. Treatment with ethanolic extract of *Asparagus racemosus* 100 mg/kg ($p<0.01$) and 200 mg/kg ($p<0.001$) *p.o.* per se for 14 consecutive days significantly reversed valproic acid-induced increase in locomotor activity of rat pups as compared to valproic acid- induced autistic pups. Treatment with standard drug diazepam (2 mg/kg *i.p*.) also decreased the valproic acid induced increased locomotor activity. The lowest dose (50 mg/kg, *p.o*.) of extract did not ($p>0.05$) affect locomotor activity of pups (Table 3).

Influence of *Asparagus racemosus* on depressive behavior of rat pups using Tail Suspension Test:

Valproic acid (500 mg/kg, *i.p*.) significantly ($p<0.001$) increased the immobility period of rat pups as compared to the control. Treatment with ethanolic extract of *Asparagus racemosus* (100 mg/kg and 200 mg/kg, *p.o*.) per se for 14 consecutive days significantly ($p<0.001$) suppressed the valproic acid- induced depressive behavior as reflected by increase in immobility period of rat pups, indicating significant anti- depressant effect similar to fluoxetine, 10 mg/kg, *i.p*. (a standard anti-depressant). The lowest dose (50 mg/kg, *p.o*.) of the extract did not significantly ($p>0.05$) affect the immobility period in rat pups (Table 4).

Effect of *Asparagus racemosus* on learning ability and memory performance of rat pups using Morris Water Maze:

In Morris Water Maze (MWM) test, rat pups search for a suitable platform as an escape attempt from repeated swimming. Valproic acid (500 mg/kg, *i.p*.) significantly ($p<0.001$) increased escape latency time on 3rd and 4th day of learning (acquisition) in Morris Water Maze (MWM) as compared to control group. Treatment with

Table 2 : Effect of ethanol extract of *Asparagus racemosus* and diazepam (standard drug) on anxiety of rat pups using Elevated Zero Maze (EZM)

Sr. No.	Treatment	Dose per kilogram	Time spent in open arm (sec)	No. of entries in open arms
1.	Control (Distilled Water)	10 ml, p.o.	62.33 ± 3.14	9.50 ± 0.61
2.	VPA	500 mg, i.p.	34.33 ± 1.20***	3.83 ± 0.47***
3.	VPA + Diazepam	2 mg, i.p.	78.83 ± 4.39###	11.50 ± 1.17
4.	VPA + *AR*	50 mg, p.o.	37.33 ± 2.10	5.33 ± 0.49
5.	VPA + *AR*	100 mg, p.o.	57.83 ± 5.87###	8.50 ± 0.56##
6.	VPA + *AR*	200 mg, p.o.	68.83 ± 1.81###	9.66 ± 0.80###
			F (5,30) = 25.44	F (5,30) = 15.74
			p<0.001	p<0.001

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 *AR* stands for *Asparagus racemosus.*
***p <0.001 as compared to vehicle treated control.
##p<0.01 and ###p< 0.001 as compared to valproic acid treated group.

Table 3 : Effect of ethanol extract of *Asparagus racemosus* and diazepam (standard drug) on locomotor activity of rat pups using actophotometer

Sr. No.	Treatment	Dose per kilogram	Locomotor activity Score
1.	Control (Distilled Water)	10 ml, p.o.	212.67 ± 4.74
2.	VPA	500 mg, i.p.	300.33 ± 3.44***
3.	VPA + Diazepam	2 mg, i.p.	169.83 ± 1.30###
4.	VPA + *AR*	50 mg, p.o.	293.83 ± 3.22
5.	VPA + *AR*	100 mg, p.o.	280.17 ± 3.13##
6.	VPA + *AR*	200 mg, p.o.	235.33 ± 3.13###

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 *AR* stands for *Asparagus racemosus.*
F (5, 30) = 242.21, p< 0.0001.
***p <0.001 as compared to vehicle treated control.
##p< 0.01 and ###p< 0.001 as compared to valproic acid treated group.

highest dose of ethanolic extract of *Asparagus racemosus* (200 mg/kg, *p.o.*) per se for 14 consecutive days significantly ($p<0.05$) reversed valproic acid- induced increase in escape latency of rat pups. Treatment with standard drug donepezil (0.75 mg/kg, *i.p.*) also significantly ($p<0.001$) reversed valproic acid- induced increase in escape latency of rat pups. Treatment with *Asparagus racemosus* extract (100 mg/kg, *p.o.*) for 14 consecutive days significantly increased the escape latency of autistic pups on 2nd, 3rd and 4th days ($p<0.01$, $p<0.001$ and $p<0.001$, respectively) in MWM. The lowest dose (50 mg/kg, *p.o.*) of the extract did not significantly ($p>0.05$) affect the escape latencies on any day of learning in MWM (Table 5a).

Time spent in target quadrant (TSTQ) indicated memory performance of rat pups. More the TSTQ, higher is the memory quotient. On 5th day (retention or memory) in Morris Water Maze (MWM) test, Valproic acid (500 mg/kg, *i.p.*) significantly ($p<0.001$) decreased the time spent in target quadrant (TSTQ) as compared to control group. Treatment with ethanolic extract of *Asparagus racemosus* (100 mg/kg and 200 mg/kg, *p.o.*) per se significantly ($p<0.001$) increased the time spent in target quadrant of autistic pups. Treatment with standard drug donepezil (0.75 mg/kg, *i.p.*) also significantly ($p<0.001$) increased the TSTQ of autistic pups. The lowest dose (50 mg/kg, *p.o.)* of extract did not significantly ($p>0.05$) affect the time spent in target quadrant (Table 5b).

Toxicity studies:

Toxicity studies were carried out as per OECD guidelines in order to ascertain safety of the plant selected.

Shatavari was found to be non-toxic even at the dose range of 500- 2000mg/kg, *p.o.*

Autism spectrum disorder (ASD) covers a range

Table 4 : Effect of ethanol extract of *Asparagus racemosus* and fluoxetine (standard drug) on immobility periods of rat pups using Tail Suspension Test (TST)

Sr. No.	Treatment	Dose per kilogram	Immobility period (sec)
1.	Control (Distilled Water)	10 ml, p.o.	197.83 ± 2.78
2.	VPA	500 mg, i.p.	261.00 ± 3.54***
3.	VPA + Fluoxetine	10 mg, i.p.	100.83 ± 3.42###
4.	VPA + *AR*	50 mg, p.o.	242.67 ± 3.81
5.	VPA + *AR*	100 mg, p.o.	212.17 ± 6.10###
6.	VPA + *AR*	200 mg, p.o.	158 ± 5.11###

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 *AR* stands for *Asparagus racemosus.*
F (5, 30) = 187.16, p< 0.0001
***p <0.001 as compared to vehicle treated control.
###p< 0.001 as compared to valproic acid treated group.

Table 5a: Effect of ethanol extract of *Asparagus racemosus* and donepezil (standard drug) on learning of rat pups using Morris Water Maze

			Morris Water Maze (Acquisition)			
Sr. No.	Treatment	Dose per kg	EL (Day 1)	EL (Day 2)	EL (Day 3)	EL (Day 4)
1.	Control (Distilled Water)	10 ml, p.o.	51.66 ±4.14	43.04 ±3.21	28.08 ±1.66	17.75±0.97
2.	VPA	500 mg, i.p.	56.29 ±1.54	51.08 ±1.32	43.66 ±1.79***	37.79±1.96***
3.	VPA + Donepezil	0.75 mg, i.p.	42.40±2.31##	35.08±1.47###	24.50±1.14###	15.54±1.03###
4.	VPA + *AR*	50 mg, p.o.	54.16±1.96	51.60±1.29	47.95±1.77	33.22±3.06
5.	VPA + *AR*	100 mg, p.o.	51.87±1.52	42.29±1.86#	31.50±0.52###	22.33±0.25###
6.	VPA + *AR*	200 mg, p.o.	46.20±0.85#	36.95±1.63###	27.37±1.51###	18.58±0.92###
			F(5,30) = 5.05	F(5,30) = 12.94	F(5,30) = 42.67	F(5,30)= 31.02
			p= 0.0018	p<0.0001	p<0.0001	p<0.0001

n= 6 in each group
Data are expressed as Mean ± SEM and analyzed by One- Way ANOVA followed by Tukey's test.
EL means Escape latency
VPA stands for Valproic acid and AR stands for Asparagus racemosus.
***p< 0.001 as compared to vehicle treated control.
#p< 0.05, ##p< 0.01 and ###p< 0.001 as compared to valproic acid treated group.

Table 5b : Effect of ethanol extract of *Asparagus racemosus* and donepezil (standard drug) on memory of rat pups using Morris Water Maze

		Morris Water Maze (Retention)	
Sr. No.	Treatment	Dose per kilogram	Time spent in target quadrant in sec (TSTQ)
1.	Control (Distilled Water)	10 ml, p.o.	31.75 ± 0.58
2.	VPA	500 mg, i.p.	20.05 ± 0.79***
3.	VPA + Donepezil	0.75 mg, i.p.	38.20 ± 0.46###
4.	VPA + *AR*	50 mg, p.o.	21.08 ± 0.82
5.	VPA + *AR*	100 mg, p.o.	25.88 ± 0.74###
6.	VPA + *AR*	200 mg, p.o.	30.08 ± 0.72###

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 *AR* stands for *Asparagus racemosus.*
F (5, 30) = 97.51, p< 0.0001.
***p <0.001 as compared to vehicle treated control.
###p< 0.001 as compared to valproic acid treated group.

of neurodevelopmental disorders occurring in early childhood as reflected by behavioral and cognitive abnormalities lasting throughout a person's life (Rapin and Katzman, 1998). ASD is categorized into 4 different types- Autistic disorder, Pervasive development disorder, not otherwise specified (PDD-NOS), Asperger's syndrome and Childhood Disintegrative disorder (Faridi and Khosrowabadi, 2017). Autism is known as a "Spectrum" disorder because there is wide variation in the type and severity of symptoms children experience. Core symptoms of autism include abnormal interpersonal and social interactions, defective communication and stereotypic behavior (Wagner *et al*., 2006). In recent years, prevalence of autism has enhanced considerably owing to chronic stress, urbanization, substance abuse, environmental pollution, hectic travel, ambitious and defective lifestyle. It is a complex neurological disorder with no clear-cut identified structural abnormality or biomarker in brain. The autistic disorder is incurable affecting quality of life of affected children and their families. However, clinical treatment available on date aims at providing symptomatic relief only (Myers and Johnson, 2007). But these allopathic medicines have severe ill- effects. Therefore, herbal plants are being explored as an alternative treatment approach. India has a huge reservoir of medicinal plants. In the light of above, we were interested to investigate the beneficial effect of Asparagus racemosus, commonly known as Shatavari in the management of autistic disorder.

Sodium valproate-induced autism in rodents is a well established experimental model to evaluate the effects of new drugs on autism (Sandhya *et al.*, 2012 and Wagner *et al.*, 2006). Female pregnant rats exposed to sodium valproate on 13th day of gestation show several anatomical abnormalities in the brain stem and cerebellum (Ingram *et al.*, 2000) of their offsprings after delivery resembling their human counterpart as found in autopsy and brain imaging studies of autistic children. These autistic pups exhibit behavioral abnormalities including higher sensitivity to pain and non-painful stimuli, repetitive and stereotypic behavior combined with lower exploratory activity, decreased number of social interactions and increased anxiety in a novel environment (Schneider and Przewlocki, 2005 and Kim *et al.*, 2011). Valproic acid exposure during neuronal development might cause dysregulation of enkephalinergic system and proenkephalin levels, leading to altered sensitivity to pain (Schneider *et al.*, 2007). The mechanisms underlying the effects of prenatal valproic acid on fetal brain development may be neural inflammation and gene regulation. Immunological changes have been reported in offsprings of valproic acid -treated mice (Schneider *et al.*, 2008). Embryonic day 12 is the critical period in rats, when valproate exposure has prominent effects for inducing the altered social behavior similar to human autistic behavior (Kim *et al.*, 2011).

In the present study, valproic acid produced hyperalgesia in rat pups as reflected by decreased tail withdrawal reflex time in tail immersion test. At higher doses, Shatavari reversed this increased sensitivity to pain (hyperalgesia). Shatavari successfully antagonized the depressive as well as anxious behavior caused by valproic acid in separate experimental models at higher doses of 100mg/kg and 200 mg/kg. This finding is consistent with the reports of Dhingra and Kumar (2007) and Garabadu and Krishnamurthy (2014). *A. racemosus* extract at

higher doses also normalized locomotor activity in autistic pups. Furthermore, in the present study, valproic acid impaired learning ability as well as memory performance when tested using Morris water maze test. This fact is in line with clinical observation that autistic children show low IQ. Shatavari at higher doses restored both the learning ability and memory performance. This finding is consistent with the reports of Ojha *et al.* (2010), who demonstrated the nootropic and anti-amnesic activities of *Asparagus racemosus* in rodents. Acetylcholine is the crucial neurotransmitter responsible for acquisition of new information (learning) and retention of acquired information (memory). Biochemical estimation of cholinesterase enzyme would throw more light on effectiveness of Shatavari in memory in VPA- induced autism.

The underlying mechanism of action of Shatavari for the beneficial effect in ameliorating autistic symptoms appears to be related to its nootropic (Ojha *et al.*, 2010), anti-oxidant (Kamat *et al.*, 2000), anti-inflammatory (Plangsombat *et al.*, 2016), anti-depressant (Dhingra and Kumar, 2007), anti-anxiety (Garabadu and Krishnamurthy, 2014) and neuroprotective (Uddin *et al.*, 2016) activity. However, the modulation of various neurotransmitter levels involved in the beneficial anti-autistic effect of Shatavari needs to be investigated. Furthermore, Shatavari has been reported to promote the development of fetus and facilitate satisfactory development of neonates during and after delivery in human beings in addition to being a galactogogue (Bazzano *et al.*, 2016).

Concluding remarks:

Asparagus racemosus appears to provide a promising remedy for the management of autistic symptoms without any serious side-effects.

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