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ARTICLE *in* NEUROTOXICOLOGY AND TERATOLOGY · JANUARY 2015

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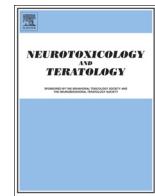


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# Inulin supplementation during gestation mitigates acrylamide-induced maternal and fetal brain oxidative dysfunctions and neurotoxicity in rats<sup>☆</sup>

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## ARTICLE INFO

### Article history:

Received 3 December 2014

Received in revised form 5 March 2015

Accepted 11 March 2015

Available online 20 March 2015

### Keywords:

Inulin  
Prebiotic  
Gestation  
Acrylamide  
Oxidative stress  
Fetus

## ABSTRACT

Accumulating evidence suggests that the developing brain is more susceptible to a variety of chemicals. Recent studies have shown a link between the enteric microbiota and brain function. While supplementation of non-digestible oligosaccharides during pregnancy has been demonstrated to positively influence human health mediated through stimulation of beneficial microbiota, our understanding on their neuromodulatory propensity is limited. In the present study, our primary focus was to examine whether supplementation of inulin (a well known fructan) during gestation can abrogate acrylamide (ACR)-induced oxidative impairments and neurotoxicity in maternal and fetal brain of rats. Initially, in a dose-determinative study, we recapitulated the impact of ACR exposure during gestation days (GD 6–19) on gestational parameters, extent of oxidative impairments in brain (maternal/fetal), cholinergic function and neurotoxicity. Subsequently, pregnant rats orally (gavage) administered with inulin (IN, 2 g/kg/day in two equal installments) supplements during gestation days (GD 0–19) were exposed to ACR (200 ppm) in drinking water. IN supplements significantly attenuated ACR-induced changes in exploratory activity (reduced open field exploration) measured on GD 14. Further, IN restored the placental weights among ACR exposed dams. Analysis of biochemical markers revealed that IN supplements effectively offset ACR associated oxidative stress not only in the maternal brain, but in the fetal brain as well. Elevated levels of protein carbonyls in maternal brain regions were completely normalized with IN supplements. More importantly, IN supplements significantly augmented the number of *Bifidobacteria* in the cecum of ACR rats which correlated well with the neurorestorative effect as evidenced by restored dopamine levels in the maternal cortex and fetal brain acetylcholinesterase activity among ACR-exposed dams. Further, IN supplements also conferred significant protection against mitochondrial dysfunction induced by ACR in both milieus. Although the precise mechanism/s by which IN supplements during pregnancy attenuate ACR induced neurotoxic impact merits further investigations, we hypothesize that it may mediate through enhanced enteric microbiota and abrogation of oxidative stress. Further, our study provides an experimental approach to explore the neuroprotective role of prebiotic oligosaccharides during pregnancy in reducing the adverse impact of developmental neurotoxicants.

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## 1. Introduction

Recent evidence has revealed that gut microbiota possesses the propensity to influence brain development and behavior (Cryan and Dinan, 2012). Epidemiological and animal studies have shown the beneficial effects of the proliferation of bacterial strains on brain chemistry (Messaoudi et al., 2011; Collins and Bercik, 2013). Administration of live

bacterial supplements (probiotics) to rodents is shown to alter trophic cytokine (brain-derived neurotrophic factor, BDNF) gene expression (O'Sullivan et al., 2011). Interestingly, probiotic cocktail reduced the process aligned with anxiety in rats and serum cortisol levels associated with psychological distress in humans (Messaoudi et al., 2011). Although the precise mechanisms mediating these effects remain unclear, the involvement of dampening down of the elevated levels of oxidative free radicals and pro-inflammatory cytokines seems likely (Cryan and O'Mahony, 2011). Hence, dietary interventions using prebiotics to stimulate the growth of beneficial intestinal microbiota is being explored as a tool to achieve various beneficial effects to the brain (Marques et al., 2014).

Fructans, such as inulin are plant derived nondigestible carbohydrates (NDO) are mainly digested by cecal microbiota (Yasuda et al.,

\* Presented in part at the Joint Meeting of the 20th Biennial Meeting of the International Society for Developmental Neuroscience (ISDN) and the 5th Annual NeuroDevNet Brain Development Conference held on 19–24 July 2014 at Montreal, Canada.

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2007) and increase the indigenous bacterial community (Kelly, 2008; van Vlies et al., 2012). Previously, supplementation of non-digestible oligosaccharides during pregnancy, has been reported to ameliorate various metabolic disorders (Qiu et al., 2008). Further, studies have demonstrated a significant diminution in the levels of procarcinogenic biomarkers in rats following prophylactic treatment with inulin (Verma and Shukla, 2013). A recent study reported the potential of inulin in alleviating the LPS-induced oxidative stress in human colon epithelial cell lines (Pasqualetti et al., 2014).

Acrylamide (ACR) is a vinyl, water-soluble alkene used in the production of glue and plastics that have various commercial applications (LoPachin, 2004; Erkekoglu and Baydar, 2014). ACR is demonstrated to be neurotoxic in humans and affects both central and peripheral nervous system. This neurotoxin has gained tremendous importance owing to its formation during frying/baking of commonly consumed foods such as fried potatoes, biscuits and coffee (JECFA, 2011). Studies in rodents have demonstrated that ACR crosses the placenta (Annola et al., 2008; von Stedingk et al., 2011) and causes reproductive/developmental toxicity coupled with adverse effects on the fetus such as lower birth weight, skeletal abnormalities (Manson et al., 2005; El-Sayyad et al., 2011) and neurodevelopmental toxicity (Takahashi et al., 2009; Ferguson et al., 2010; Garey and Paule, 2010; El-Sayyad et al., 2011). The involvement of oxidative stress and inflammatory responses in ACR-induced neurotoxicity are widely accepted (Lopachin and Gavin, 2008; Prasad and Muralidhara, 2012, 2013). Further, ACR is shown to form conjugates with reduced glutathione (GSH), and the resulting complex is metabolized by cytochrome P450 (subtype CYP 2E1) to form glycidamide. Recent evidence suggests that ACR forms adducts within presynaptic proteins resulting in altered neurotransmission and inactivation of enzymes involved in neuronal energy production (LoPachin and Gavin, 2012; Martyniuk et al., 2013).

Compelling evidence exists that chemical agents widely disseminated in the environment are important triggers of neurological anomalies (Grandjean and Landrigan, 2006, 2014). Developing brain is of particular interest since it is highly susceptible to neurotoxin insult during the critical window of vulnerability (Rice and Barone, 2000). Given the wide industrial applications and the possible neurotoxic effects associated with the chronic human exposure to ACR starting in utero, it is relevant to develop specific therapeutic strategies to abrogate ACR-induced fetal neurotoxic effects. Hence, in the present study our primary objective was to understand whether supplementation of inulin, (a widely consumed NDO) to dams during pregnancy possess the potential to attenuate ACR-induced oxidative stress and neurotoxicity in a rat model. As a prelude, we characterized some of the biochemical perturbations (oxidative impairments and neurotoxicity) in rats associated with gestational exposure to varying doses of ACR. Subsequently we investigated the ameliorative effect of inulin supplements against ACR intoxication in maternal as well as fetal brain.

## 2. Materials and methods

### 2.1. Chemicals

Acrylamide (electrophoresis grade; purity > 99%, Product #A8887), inulin from chicory (Product #I2255), 2-thiobarbituric acid, 1,1,3,3-tetramethoxypropane, 2',7'-dichlorofluorescein (DCF), 2',7'-dichlorofluorescein diacetate, N,N,N',N'-tetramethylmethylenediamine, L-glutathione reduced, acetylthiocholine iodide, S-butylthiocholine iodide, nicotinamide adenine dinucleotide, thiazolyl blue tetrazolium bromide, dopamine were procured from Sigma Aldrich, St Louis, MO, USA. 2,4-Dinitrophenylhydrazine, 1-chloro-2,4,-dinitro benzene, nicotinamide adenine dinucleotide phosphate, cytochrome c were procured from Sisco Research Laboratories Pvt. Ltd., India. All other reagents used were of analytical or HPLC grade.

### 2.2. Experimental animals

Male and female adult Wistar rats were randomly drawn from the stock colony of the institute's animal house facility ( $200 \pm 10$  g), and acclimatized for 1 week. Rats were housed in vented polypropylene cages ( $40 \times 28 \times 16$  cm $^3$ ) with dust-free shaved wood bedding. Animals housed in a light- and temperature-controlled room (14 h/10 h light-dark cycle, 21 °C, 50% humidity) were provided with commercial rodent pellet diet (Sai Durga Feeds & Foods, Bengaluru, India) and tap water ad libitum. Following acclimatization, virgin female rats were allowed to mate overnight with males (2:1 ratio). Vaginal smear using 0.9% sodium chloride was performed daily to ascertain pregnancy. The swab was examined on a slide using a bright-field microscope for the presence of spermatozoa. The presence of sperm was designated as gestation day (GD) 0, and the females were separated, weighed and individually housed. All protocols were approved by the institutional animal ethics committee in conformance with the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India. Handling, as well as care of animals during sacrifice, was strictly according to the standard guidelines of the Institutional Ethics Committee (Registration #49/1999/CPCSEA).

### 2.3. Experimental procedure

#### 2.3.1. Study 1: exposure of pregnant rats to acrylamide (ACR): Effect on gestational parameters and oxidative markers in fetal and maternal brain

Sperm-positive female rats were placed in plastic cages and randomly divided into 4 groups. While dams ( $n = 6$ ) of treatment groups (Groups II, III and IV) received 50, 100 and 200 ppm ACR in drinking water during gestation days (GD) 6 to 19, those of control group (group-I) received deionized water. Water consumption and feed intake were recorded daily while body weight gain was monitored on alternate days. On GD 19, all dams were sacrificed, maternal brains and uterine horns were rapidly exteriorized, immersed in ice-cold phosphate buffered saline (PBS), blotted dry and cryostat cooled as previously described (Shivananjappa and Muralidhara, 2012). Fetuses and placenta were separated from uterine horns after fixing it to a wax base, immersed in ice-cold PBS. Further, fetal brain were excised from fetuses, rinsed in ice-cold PBS, the meninges were removed and stored at –80 °C until further processing. Similarly, brains were excised from the dams and brain regions viz., cortex and cerebellum were separated on ice and stored until used. Markers of oxidative stress and antioxidant/detoxifying enzymes were determined in the maternal brain regions (cortex and cerebellum) and whole fetal brain.

#### 2.3.2. Study 2: protective effects of inulin supplements in the ACR model

Pregnant rats were assigned four groups and were treated as follows: Group I – Untreated controls; Group II – Inulin (IN); Group III and Group IV – ACR (200 ppm, GD 6–19) in drinking water. Rats of Groups II and IV were orally administered (gavage) with inulin (IN, 2 g/kg/day) supplements during gestation days (GD 0–19). The total daily dose of IN was given as split dose (twice a day at the rate of 1 g/kg at 09:00 and 15:00 h each day). The IN dose was selected based on our preliminary study, and the doses were adjusted daily to account for the changes in body weight of the dams. Dams of all treatment groups were subjected to open field test on GD 14 and were sacrificed on GD 19. Further procedures were identical as described in Study 1. Biochemical parameters were determined in the maternal brain (cortex and cerebellum) and fetal brain. The cecum from dams of all treatment groups was collected, and subjected to quantification of *Bifidobacteria* and *Lactobacillus* adopting standard microbiological procedures.

## 2.4. Analysis of maternal behavior in the open field test

A 90 cm<sup>3</sup> open rectangular wooden apparatus was used to assess the exploratory behavior by way of an open field test. Dams were placed individually in the corner of the arena (illuminated by ambient fluorescent lights) and permitted free exploration of the open field for 10 min, during which their movements were recorded and analyzed. Relevant behavioral indices of exploratory activity such as the frequency of entries into and total duration of time spent in a predefined center zone of the open field were recorded as previously described (Litteljohn et al., 2009). Open field test was conducted between 09:00 and 12:00 h on GD 14.

## 2.5. Sample preparation

### 2.5.1. Enumeration of cecal bacteria

For the quantification of the microbial population, the cecum from dams was excised, and weight (with cecal contents) was recorded. Samples of digesta from cecum were suitably diluted in the storage medium before storage at –20 °C. Further for bacterial enumeration by selective plating, suspensions of digesta (1 g) were diluted 10-fold using sterile saline and the resultant suspension was homogenized followed by serial dilutions. Three appropriate dilutions (100 µl each) were spread aseptically in triplicates onto selective agar: Bifidobacterium iodoacetate agar (anaerobically) and *Lactobacillus* MRS (de Mann Rogosa Sharpe) agar (aerobically), incubated at 37 °C for 24 and 48 h respectively for *Bifidobacteria* and *Lactobacilli* enumeration. Microbial counts were expressed as log colony-forming units (cfu)/g wet tissue weight.

### 2.5.2. Preparation of brain homogenates

In brief, 10% (w/v) homogenate of the fetal brain (pool of four or five brains) and maternal brain regions was prepared in ice-cold isolation buffer (100 mM sucrose, 10 mM EDTA, 100 mM Tris-HCl; pH 7.4) and centrifuged at 1000 g (10 min at 4 °C) to remove nuclei. Cytosol and mitochondria were fractionated by differential centrifugation (700 g and 4500 g) for 10 min at 4 °C (Sigma-2K16, Germany). The crude mitochondrial pellet obtained was washed gently and resuspended in 200 mM mannitol, 70 mM sucrose, 10 mM HEPES (pH 7.4) and 0.1 mM EDTA (Moreadith and Fiskum, 1984).

## 2.6. Biochemical determinations in brain tissues

### 2.6.1. Measurement of markers of oxidative stress

Reactive oxygen species (ROS) production was measured using 2',7'-dichlorodihydrofluorescein diacetate (H<sub>2</sub>DCF-DA) assay. H<sub>2</sub>DCF-DA is deesterified to polar derivative (H<sub>2</sub>DCFH) by intracellular esterases, subsequently oxidized to the fluorescent compound DCF by ROS (Gokul and Muralidhara, 2014). In brief, an aliquot (equivalent to 100 µg protein) was incubated in Locke's buffer (in mM: 154 NaCl, 5.6 KCl, 5 HEPES, 3.6 NaHCO<sub>3</sub>, 2 CaCl<sub>2</sub> and 10 glucose; pH 7.4) containing 5 µM H<sub>2</sub>DCFH-DA (10 µl) for 30 min. The fluorescent product (DCF) was measured using a spectrofluorimeter with excitation and emission wavelengths of 480 nm and 530 nm respectively. The ROS generation was calculated by comparison to a standard curve of DCF and expressed as pmol DCF/min/mg protein.

Hydroperoxide (HP) levels were measured by a modification of the ferrous oxidation-xenol orange (FOX 1 reagent) assay as previously described (Wolff, 1994). The assay is based on the oxidation of ferrous to ferric ions under acidic conditions. Samples of aliquot of cytosol (100 µg protein) were added to 1 mL FOX reagent (containing 100 µM xenol orange; 250 µM ammonium ferrous sulfate; 100 µM sorbitol and 25 mM H<sub>2</sub>SO<sub>4</sub>) and incubated at room temperature for 30 min. Stable complex of ferric ions formed with the indicator dye xenol orange was measured at 560 nm using a spectrophotometer. The concentration of HP was calculated using the molar extinction co-

efficient ( $\epsilon = 2.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ ) and expressed as nmol HP/mg protein.

As an index of lipid peroxidation (LPO), the formation of thiobarbituric acid reactive substances (TBARS) was measured as described previously (Ohkawa et al., 1979). In brief the reaction mixture contained an aliquot of cytosolic fraction of different brain regions (500 µg protein), 1.5 mL of acetic acid solution (2.5 M HCl, pH 3.5), 1.5 mL of thiobarbituric acid (0.8% w/v) and 0.2 mL sodium dodecylsulfate (8% w/v). The mixture was heated to 95 °C for 45 min and after cooling to room temperature, the pink chromogene was extracted with 1-butanol and measured at 532 nm quantified as malondialdehyde (MDA) equivalents using 1,1,3,3-tetramethoxypropane as the standard.

### 2.6.2. Determination of protein carbonyls

Levels of protein carbonyls (PC) were determined as described previously (Levine et al., 1990) with minor modifications. Briefly, an aliquot of sample (500 µg protein) of cytosol was incubated with 2,4-dinitrophenyl hydrazine (10 mM in 2 N HCl), while shaking intermittently for 1 h at room temperature. After incubation, the protein was precipitated by addition of trichloroacetic acid (20%) and then centrifuged. The pellet was washed with acetone, and the final precipitate was dissolved in Tris-HCl buffer (2 mM, pH 7.4 containing 200 mM NaCl and 2% SDS). The absorbance was measured at 370 nm and expressed as nmol carbonyl/mg protein ( $\epsilon = 22,000 \text{ M}^{-1} \text{ cm}^{-1}$ ).

### 2.6.3. Reduced glutathione (GSH) levels

Reduced GSH content was quantified by fluorescence detection after reaction of the aliquots of test sample containing CH<sub>2</sub>O<sub>2</sub>/NaH<sub>2</sub>PO<sub>4</sub>-EDTA, with O-phthalaldehyde (OPT) (Mokrasch and Teschke, 1984). In brief, cytosol (0.1 mg protein) was resuspended in 1.5 mL phosphate buffer (0.1 M NaH<sub>2</sub>PO<sub>4</sub>, 5 mM EDTA, pH 8.0) and 500 µL formic acid (0.1 M) was rapidly centrifuged at 10,000 g for 10 min. 100 µL of supernatant was added to 1.8 mL phosphate buffer followed by 100 µL OPT (1 mg/mL in methanol). After thorough mixing and incubation for 45 min in the dark, fluorescence was measured with excitation at 345 and emission at 425 nm. The concentration of GSH was calculated from the standard curve and expressed as µg GSH/mg protein.

### 2.6.4. Activity levels of antioxidant enzymes

Superoxide dismutase (SOD) activity was assayed by monitoring the inhibition of quercetin auto-oxidation. In brief, the assay mixture contained a total volume of 1 mL reaction mixture with sample (3–5 µg protein), 0.016 M sodium phosphate buffer (pH 7.8), 8 mM TEMED and 0.08 mM ethylenediaminetetraacetic acid (EDTA) and quercetin (1.5 mg/ml dimethyl formamide). Reaction was monitored at 406 nm for 3 min, expressed as the amount of protein required to inhibit 50% of quercetin auto-oxidation (Kostyuk and Potapovich, 1989).

Catalase (CAT) activity was determined by the method described previously (Aebi, 1984). An aliquot of the sample (10 µg protein equivalent) was added to 1 mL reaction mixture containing 50 µL of H<sub>2</sub>O<sub>2</sub> (10 mM), 900 µL sodium-phosphate buffer (0.1 mM, pH 7.0) to initiate the reaction. The decrease in H<sub>2</sub>O<sub>2</sub> was monitored at 240 nm for 3 min and expressed as nmol H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein ( $\epsilon = 43.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

### 2.6.5. Phase II detoxifying enzyme activity assays

Glutathione peroxidase (GPx) activity was assayed using H<sub>2</sub>O<sub>2</sub> as a substrate coupled with GR-catalyzed oxidation of NADPH (Flohe and Gunzler, 1984). Reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM EDTA, 1 mM GSH, 0.24 U glutathione reductase and 0.1 mg of cytosol, incubated for 10 min at 37 °C. Reaction was initiated by the addition of 0.15 mM NADPH, 1 mM NaN<sub>3</sub> and monitored for 3 min at 340 nm. Then 0.1 mg homogenate and 0.15 mM H<sub>2</sub>O<sub>2</sub> were added and the decrease in absorbance was

measured. Results were expressed as nmoles of NADPH oxidized/min/mg protein.

Thioredoxin reductase (TRR) activity in the sample was measured by monitoring the DTNB reduction at 412 nm (Luthman and Holmgren, 1982). Briefly, the reaction mixture contained test sample in 0.1 M potassium phosphate buffer (in mM: 10 EDTA, 0.2 NADPH; pH 7.0). The activity was expressed as nmol substrate reduced/min/mg protein ( $\epsilon = 13.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

Glutathione S-transferase (GST) activity was analyzed by monitoring the conjugation of glutathione to chloro-2,4-dinitrobenzene (CDNB) (Guthenberg et al., 1985). The reaction was started by adding a cytosolic aliquot (0.01 mg protein) to the phosphate buffer (0.1 M, pH 6.5) containing 0.5 mM EDTA, 0.075 CDNB, 0.05 mM GSH. The increase in the optical density was monitored for 3 min at 340 nm, and the data were expressed as nmol conjugate formed/min/mg protein ( $\epsilon = 9.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

## 2.7. Cholinergic function

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities were determined as described earlier (Ellman et al., 1961). The enzyme activities were expressed as nmoles substrate hydrolyzed/min/mg protein.

## 2.8. HPLC determination of dopamine (DA) concentration

Levels of DA were determined in the cytosol of fetal brain and maternal cortex via HPLC (Dalmiaz et al., 2007). Briefly, deproteinized tissue supernatants (20  $\mu\text{l}$  samples) were passed through the column (5 mm, C18 reverse phase, 150 mm  $\times$  4.6 mm) connected to a UV detector (SPD-10 Model Avp). A flow rate of 1 ml/min (1400–1600 p.s.i) was maintained at using a Waters M-6000 pump. The mobile phase comprised of 0.2% trifluoroacetic acid and methanol (70:30, v/v; pH 3.5) in deionized water, subsequently filtered (0.22 mm filter paper) and degassed. Determination of the area and height of the peaks were carried out using an integrator. DA levels were calculated using a standard curve and expressed as  $\mu\text{g DA/g tissue}$ .

## 2.9. Mitochondrial enzymes

NADH-cytochrome c reductase (complexes I–III) activity was measured by adding an aliquot of mitochondrial sample (50  $\mu\text{g}$  protein) to a reaction medium containing phosphate buffer (0.1 M, pH 7.4), 0.2 mM NADH and 1 mM KCN. The reaction was initiated by the addition of oxidized cytochrome c (0.1 mM) and decrease in absorbance was monitored for 3 min at 550 nm. Enzyme activity was expressed as nmol cytochrome C reduced/min/mg protein ( $\epsilon = 19.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ) (Navarro et al., 2004).

MTT reduction was determined by a modification of the method described earlier (Berridge and Tan, 1993). Briefly, mitochondrial samples (equivalent to 10  $\mu\text{g}$  protein) were incubated in a mannitol-sucrose–HEPES buffer (pH 7.4) containing sodium succinate (20 mM) and 10  $\mu\text{l}$  MTT (5 mg/ml) for 60 min at 37 °C and MTT reduction was stopped by addition of 1 ml of buffer, 10% sodium dodecyl sulfate (SDS): 45% N,N'-dimethylformamide (DMF) [v/v, pH 4.8]. The extent of MTT reduction to formazan was determined by measuring the optical density at 570 nm and results were expressed as OD/mg protein.

## 2.10. Protein estimation

Protein concentrations were determined (in cytosolic/mitochondrial fractions) by the method of Lowry et al., 1951, using bovine serum albumin as a standard.

## 2.11. Statistical data analysis

All data were analyzed by one-way ANOVA followed by a post hoc Dunnett's and Tukey tests for comparisons ( $p < 0.05$ ) where appropriate. Data were evaluated using SPSS statistical software package (version 17.0).

## 3. Results

### 3.1. Exposure of pregnant rats to ACR

#### 3.1.1. Effects of ACR on gestational parameters

Daily food and water intake among rats consuming ACR in water (low dose – 50 ppm; mid dose – 100 ppm; and high dose – 200 ppm) were highly comparable to those of untreated controls (data not shown). Further, there was no significant effect on the body weights of dams that consumed ACR in drinking water at any of the dosages (Table 1). ACR exposure caused no significant changes in the number of implants, while significant reduction in the placental weight (high dose – 20%) and fetal body weight (mid dose – 19% and high dose – 36%) was observed. The cumulative ACR intake was arrived at by averaging the water intake for the first 7 days (GD 6–12) and the second seven days (GD 13–19) for each rat. The mean intake of ACR among the three groups was: – 1.91, 3.71 and 6.94 mg/rat/day at the doses of 50, 100 and 200 ppm respectively.

#### 3.1.2. ACR induced oxidative stress in the maternal brain (MB) and fetal brain (FB)

Since none of the biochemical parameters were significantly altered at the low dose of ACR, data has not been represented at this dose. While only a marginal effect was evident at the mid-dose, ACR at the high dose was effective in inducing oxidative stress in both maternal and fetal milieu. Significantly elevated levels of ROS were noticeable in MB regions (cortex: 21%; cerebellum: 27%) and FB (60%) (Fig. 1A). While MDA levels (an indicator of lipid peroxidation) were markedly elevated in MB regions (cortex: 21% and cerebellum: 26%) and FB (37%) (Fig. 1B), the basal levels of HP were significantly enhanced only in MB cerebellum (25%) and FB (24%) (Fig. 1C).

#### 3.1.3. Effect of ACR exposure on antioxidant and phase II detoxifying enzyme activities

ACR at high dose caused significant diminution in the activity levels of CAT in MB cerebellum (20%) and FB (18%) (Table 2). Further, ACR reduced the SOD activity only in MB cerebellum (20%). Likewise, GPx activity significantly decreased in MB cerebellum (19%) and FB (24%). However, ACR exposure at both the doses significantly enhanced the activity levels of GST (mid dose: MB cortex – 27%, FB – 18%; high dose: MB cortex – 29% and cerebellum – 27%, FB: 23%).

#### 3.1.4. Effect on cholinergic function

At the high dose, ACR caused enhancement in the activity levels of AChE in MB regions (cortex: 34%; cerebellum: 44%) and FB (37%) (Fig. 2A). Similarly, the activity of nonspecific cholinesterase (BuChE) was also enhanced markedly in MB regions (cortex: 48%; cerebellum: 55%), but marginally in FB (Fig. 2B).

### 3.2. Protective effects of oral inulin (IN) supplements against ACR-induced neurotoxicity

#### 3.2.1. Modulatory effect of IN on exploratory behavior of dams – open field test

ACR exposure significantly affected the exploratory activity among dams as reflected by a marked (52%) reduction in the number of the center zone entries (CTR vs. ACR,  $5.2 \pm 0.5$  vs.  $2.5 \pm 0.3$ ). However, ACR dams supplemented with IN displayed more exploration of the center zone of the arena relative to their ACR alone counterparts

**Table 1**

Body weight changes, placental weights and fetal weights of dams exposed to acrylamide (ACR) in drinking water.

Parameter	ACR (ppm)			
	0	50	100	200
Body weight (g) on GD 6	211.1 ± 5.34	209.4 ± 4.40	212.5 ± 8.43	214.7 ± 8.33
Body weight (g) on GD 19	261.4 ± 9.44	258.6 ± 8.51	255.2 ± 12.8	248.1 ± 11.3
Number of implants/liter <sup>a</sup>	11.5 ± 2.10	12.2 ± 0.68	11.3 ± 3.22	10.8 ± 2.82
Placental weight/liter (g)	0.55 ± 0.04	0.58 ± 0.03	0.51 ± 0.05	0.44 ± 0.02*
Fetal weight/liter (g)	3.37 ± 0.27	3.43 ± 0.25	2.73 ± 0.53*	2.13 ± 0.24*

ACR (200 ppm) was provided during gestation days (GD) 6–19.

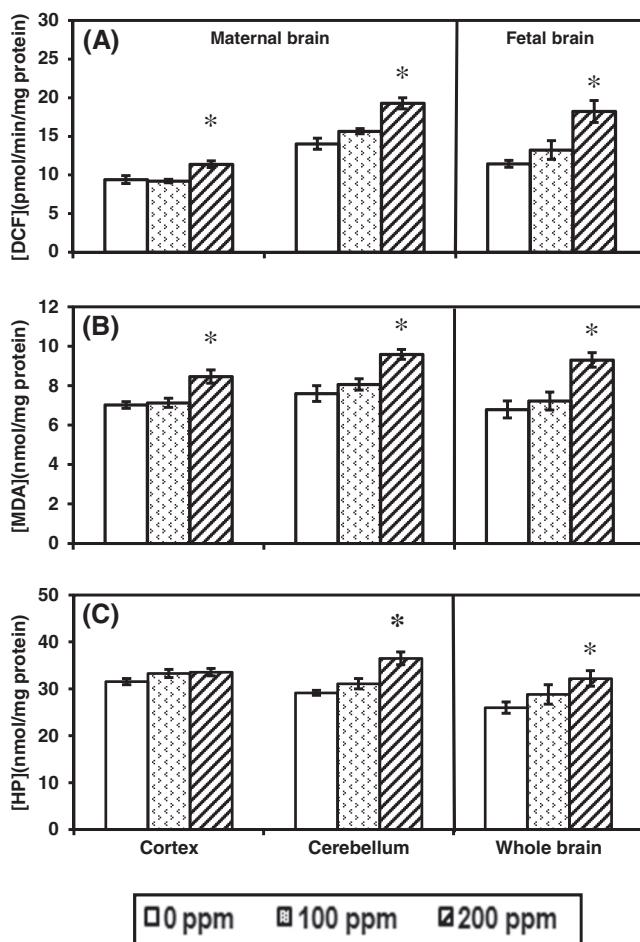
Results are expressed as mean ± SE ( $n = 6$ /group). Data were analyzed by one-way ANOVA followed by post hoc Dunnett's test. \* Significant against 0 ppm at  $p < 0.05$ .

<sup>a</sup> One dead implant at ACR 200 ppm.

(Fig. 3A). Further, ACR exposed rats spent relatively lesser duration of time (CTR vs. ACR,  $8.3 \pm 0.5$  vs.  $6.1 \pm 0.3$  s) exploring the center of the open field, however, IN supplementation had no significant impact on the time spent among ACR exposed rats as depicted in Fig. 3B.

### 3.2.2. Effect of IN on placental and fetal weights

As summarized in Table 3, no significant changes occurred in the number of implants with ACR exposure and those which received IN per se. Interestingly, dams that received IN showed significantly restored placental weight compared to those dams that received ACR alone (ACR vs. IN + ACR,  $0.40 \pm 0.06$  vs.  $0.48 \pm 0.03$ ). However, IN supplements did not influence the ACR-induced reduced fetal body weights.



**Fig. 1.** Effect of acrylamide (ACR; GD 6–19) exposure in drinking water on oxidative markers viz., reactive oxygen species (A), malondialdehyde (B) and hydroperoxide (C) levels in maternal brain regions (cortex and cerebellum) and fetal brain. Values are mean ± SE ( $n = 6$ ). Data analyzed by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test. \*Significant against 0 ppm at  $p < 0.05$ .

### 3.2.3. IN supplements abrogate ACR induced oxidative stress in MB and FB regions

IN supplements significantly attenuated ACR induced biochemical alterations (oxidative stress markers, GSH and protein carbonyl levels) in maternal and fetal brains (Table 4). Exposure to ACR enhanced ROS levels in MB cortex and cerebellum, whereas IN supplementation appeared significantly to suppress the elevated levels of oxidative stress marker only in the cortex. Similarly, the elevated ROS levels in the FB were also significantly diminished among IN supplemented ACR dams compared to those unsupplemented ACR dams. Further, ACR induced elevation in MDA levels was completely offset in both the cortex and cerebellum among dams supplemented with IN. Although GSH levels were marginally reduced in MB and FB region, IN supplements did not appreciably affect the levels. Consistent with the FB, ACR induced enhanced levels of PC were normalized in both the maternal brain regions among dams provided IN supplements.

### 3.2.4. Modulatory effect of IN on antioxidant enzymes and glutathione s-transferase

ACR exposure diminished the activity of SOD only in MB cerebellum that was restored by IN supplements (Fig. 4A). Interestingly, the FB among ACR dams supplemented with IN showed markedly enhanced SOD activity. Likewise, ACR significantly reduced CAT activity in FB and IN supplementation prevented this ACR-induced effect (Fig. 4B). Further, diminished activity levels of TrxR in MB cortex (43%) and cerebellum (25%) was also significantly attenuated by IN supplements

**Table 2**

Effect of acrylamide (ACR) exposure on the activities of antioxidant enzymes and glutathione s-transferase in maternal brain regions and fetal brain.

Brain region/ACR (ppm)	CAT <sup>a</sup>	SOD <sup>b</sup>	GPx <sup>c</sup>	GST <sup>d</sup>
<i>Maternal brain</i>				
Cortex				
0	2.65 ± 0.10	159.3 ± 4.30	26.8 ± 0.77	51.4 ± 2.43
100	2.76 ± 0.11	160.0 ± 3.78	25.6 ± 1.06	65.2 ± 1.07*
200	2.42 ± 0.09	145.3 ± 7.43	24.7 ± 0.73	66.4 ± 3.94*
Cerebellum				
0	5.57 ± 0.07	163.9 ± 6.44	23.3 ± 0.94	69.6 ± 1.52
100	5.31 ± 0.25	153.5 ± 7.60	22.4 ± 0.32	75.6 ± 1.42
200	4.48 ± 0.14*	121.4 ± 9.45*	18.8 ± 0.77*	88.2 ± 2.56*
<i>Fetal brain</i>				
0	12.5 ± 0.73	150.8 ± 8.15	42.2 ± 2.25	55.8 ± 0.54
100	12.1 ± 1.35	153.3 ± 9.25	39.4 ± 2.60	65.9 ± 1.20*
200	10.2 ± 0.47*	152.9 ± 5.05	32.1 ± 1.85*	68.8 ± 2.19*

ACR (200 ppm) was provided in drinking water during gestation days (GD) 6–19. Results are expressed as mean ± SE ( $n = 6$ /group). Data analyzed by one-way ANOVA followed by post hoc Dunnett's test.

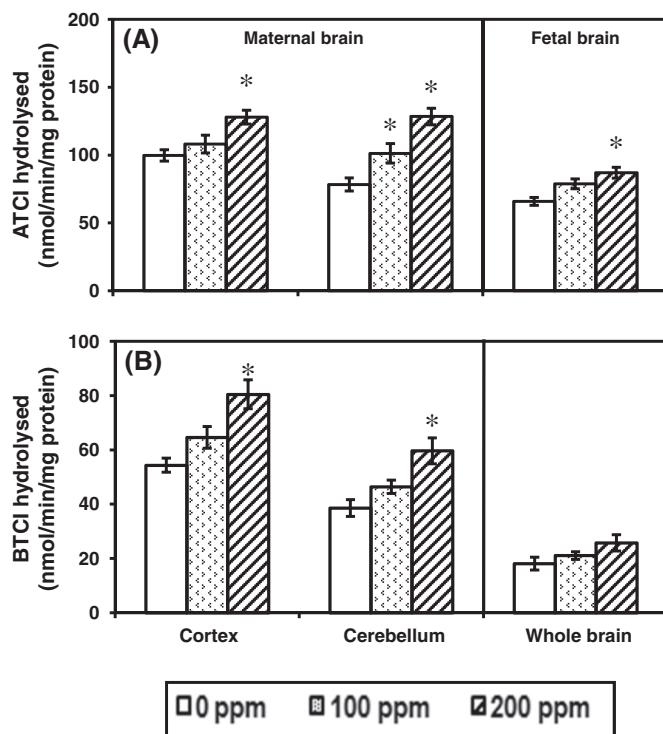
<sup>a</sup> Catalase, nmol hydrogen peroxide decomposed/min/mg protein.

<sup>b</sup> Superoxide dismutase, Units/mg protein.

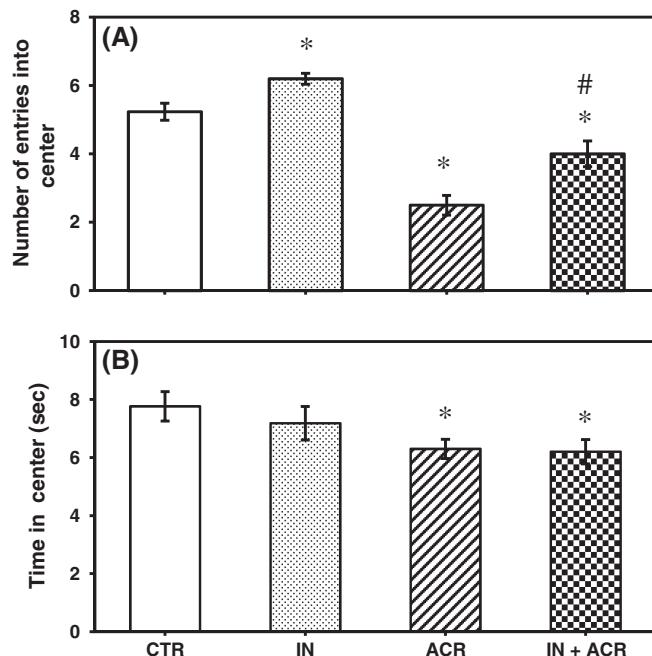
<sup>c</sup> Glutathione peroxidase, nmol NADPH oxidized/min/mg protein.

<sup>d</sup> Glutathione s transferase, nmol conjugate formed/min/mg protein.

\* Significant against 0 ppm at  $p < 0.05$ .



**Fig. 2.** Effect of acrylamide (ACR) exposure in drinking water (GD 6–19) on the activities of acetylcholinesterases (A) and butyrylcholinesterase (B) in maternal brain regions (cortex and cerebellum) and fetal brain. Values are mean  $\pm$  SE ( $n = 6$ ). Data analyzed by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test. \*Significant against 0 ppm at  $p < 0.05$ .



**Fig. 3.** Exploratory behavior in an open field environment. Modulatory effect of IN supplementation (GD 0–19) on indices of exploratory behavior: number of entries into central area of an open field arena (A) and time spent in the central area (B) among dams exposed to acrylamide (ACR) in drinking water (GD 6–19). Results are expressed as mean  $\pm$  SE ( $n = 6$ ). Data analyzed by one-way ANOVA followed by post hoc Tukey test ( $p < 0.05$ ). \*Significant against control; #significant against ACR.

**Table 3**

Modulatory effect of inulin (IN) supplementation on gestational parameters among dams exposed to acrylamide (ACR).

Parameter	Control	IN	ACR	IN + ACR
Number of implants/liter <sup>a</sup>	11.3 $\pm$ 0.04	12.8 $\pm$ 0.3	11.1 $\pm$ 0.07	11.7 $\pm$ 0.06
Placental weight/liter (g)	0.53 $\pm$ 0.03	0.52 $\pm$ 0.02	0.40 $\pm$ 0.06*	0.48 $\pm$ 0.03#
Fetal weight/liter (g)	3.20 $\pm$ 0.04	3.31 $\pm$ 0.03	2.21 $\pm$ 0.07*	2.58 $\pm$ 0.06

IN (2 g/kg bw/day) supplements were provided during GD 0–19; ACR (200 ppm) was provided in drinking water during gestation days (GD) 6–19.

Results are expressed as mean  $\pm$  SE ( $n = 6$ /group). Data analyzed by one-way ANOVA followed by post hoc Tukey test ( $p < 0.05$ ).

\* Significant against control.

# Significant against ACR.

(Fig. 4C). However, ACR enhanced activity levels of GST in MB and FB, were further elevated with IN supplementation (Fig. 4D).

### 3.2.5. Effect of IN on AChE activity and dopamine (DA) levels

While ACR induced a significant elevation in the activity levels of AChE in MB regions, IN supplementation partially restored the activity levels in the cerebellum (Fig. 5A). Likewise, FB of IN + ACR treated dams showed a marginal restoration of the enzyme activity. ACR exposure significantly depleted (19%) DA levels in MB cortex, while no effect was noticed in FB. However, with IN supplementation the levels of monoamine were restored in cortex (Fig. 5B).

### 3.2.6. IN rescues mitochondrial dysfunction induced by ACR

ACR exposure caused a significant decrease in the activity of complexes I-II only in MB (cortex: 29% and cerebellum: 22%) and IN supplementation attenuated the activity levels (Fig. 6A). Further, reduction in MTT levels in MB cerebellum among ACR dams was restored to normalcy with IN supplementation (Fig. 6B).

### 3.2.7. IN supplementation alters rat cecal characteristics

Data on the cecal wet weight, *Bifidobacteria* and *Lactobacteria* numbers among control and treatment groups is presented in Table 5. Total wet weight of the cecum showed no significant change with either ACR and/or IN supplements. However, ACR exposure significantly

**Table 4**

Effect of inulin (IN) supplementation on oxidative markers in maternal brain regions (cortex and cerebellum) and fetal brain among acrylamide (ACR) exposed dams.

	ROS <sup>a</sup>	MDA <sup>b</sup>	GSH <sup>c</sup>	PC <sup>d</sup>
<i>Maternal brain</i>				
Cortex				
Control	8.40 $\pm$ 0.52	7.02 $\pm$ 1.54	14.3 $\pm$ 0.32	13.8 $\pm$ 0.37
IN	9.48 $\pm$ 0.70	5.13 $\pm$ 1.77*	14.7 $\pm$ 0.54	13.2 $\pm$ 0.80
ACR	10.38 $\pm$ 0.33*	9.08 $\pm$ 0.60*	11.7 $\pm$ 0.19	18.6 $\pm$ 0.45*
IN + ACR	8.40 $\pm$ 0.50#	7.12 $\pm$ 1.48#	13.2 $\pm$ 0.65	14.1 $\pm$ 1.22#
Cerebellum				
Control	12.2 $\pm$ 0.40	7.14 $\pm$ 1.35	13.1 $\pm$ 1.18	14.3 $\pm$ 0.32
IN	12.6 $\pm$ 0.27	6.86 $\pm$ 0.78	12.2 $\pm$ 0.6	14.5 $\pm$ 0.54
ACR	15.8 $\pm$ 0.75*	9.11 $\pm$ 0.30*	12.5 $\pm$ 0.42	18.7 $\pm$ 0.19*
IN + ACR	14.2 $\pm$ 0.25	7.33 $\pm$ 0.51#	11.8 $\pm$ 0.80	13.2 $\pm$ 0.65#
<i>Fetal brain</i>				
Control	10.4 $\pm$ 0.44	6.79 $\pm$ 0.53	8.8 $\pm$ 0.37	17.3 $\pm$ 0.82
IN	7.92 $\pm$ 0.85*	5.72 $\pm$ 0.72	8.6 $\pm$ 0.40	13.6 $\pm$ 0.70*
ACR	15.0 $\pm$ 0.60*	9.50 $\pm$ 0.37*	8.0 $\pm$ 0.45	21.4 $\pm$ 0.65*
IN + ACR	11.1 $\pm$ 0.52#	7.15 $\pm$ 0.34#	8.1 $\pm$ 1.22	18.4 $\pm$ 0.48#

IN (2 g/kg bw/day) supplements were provided during gestation days (GD) 0–19; ACR (200 ppm) was provided in drinking water during GD 6–19.

Results are expressed as mean  $\pm$  SE ( $n = 6$ /group). Data were analyzed by one-way ANOVA followed by post hoc Tukey test ( $p < 0.05$ ).

\* Significant against control.

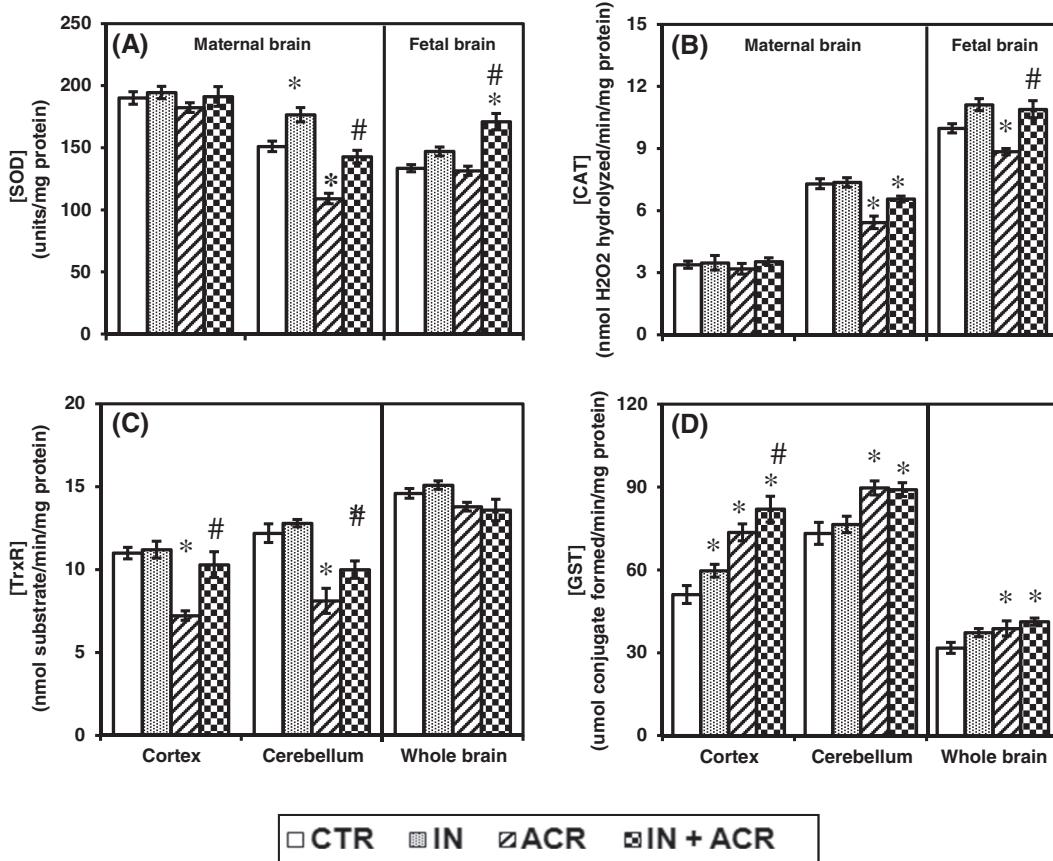
# Significant against ACR.

<sup>a</sup> Reactive oxygen species, nmol DCF/min/mg protein.

<sup>b</sup> Malondialdehyde, nmol MDA/min/mg protein.

<sup>c</sup> Reduced glutathione,  $\mu$ g GSH/mg protein.

<sup>d</sup> Protein carbonyls, nmol carbonyls/mg protein.



**Fig. 4.** Modulatory effect of gestational IN supplementation (GD 0–19) on the activities of superoxide dismutase (A), catalase (B), thioredoxin reductase (C) and glutathione S transferase (D) in maternal brain regions (cortex and cerebellum) and fetal brain among dams exposed to acrylamide (ACR, GD 6–19). Results are expressed as mean  $\pm$  SE ( $n = 6$ ). Data analyzed by one-way ANOVA followed by post hoc Tukey test ( $p < 0.05$ ). \*Significant against control; #significant against ACR.

lowered (15%) the number of *Bifidobacteria* in the cecum and followup comparisons did reveal a significant increase in the numbers among ACR dams supplemented with IN (ACR vs. IN + ACR,  $8.23 \times 10^8 \pm 0.04$  vs.  $9.16 \times 10^8 \pm 0.06$ ,  $p < 0.05$ ).

### 3.3. Correlation between cecal *Bifidobacteria* and DA levels

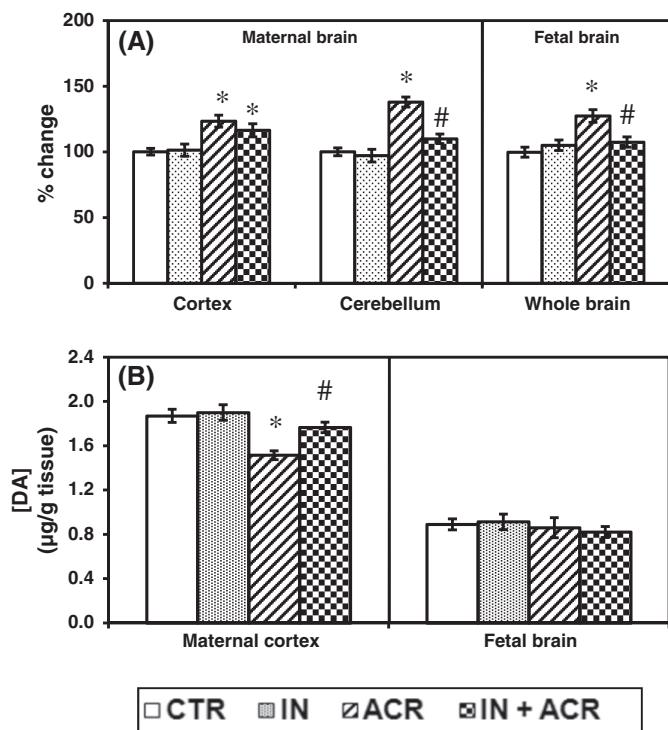
Pearson's correlation analysis revealed a significant correlation in cecal *Bifidobacteria* number and DA levels in maternal cortex (Pearson's  $r = 0.59$ ,  $p < 0.05$ ) in the analysis of ACR and IN + ACR treated groups. However, in contrast no such correlation was evident with cecal bacterial numbers and fetal brain DA levels.

## 4. Discussion

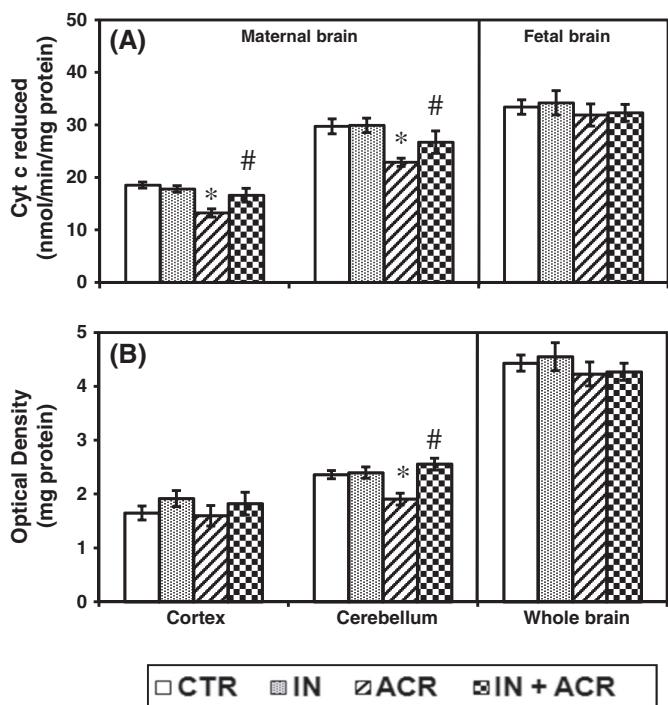
Recent evidence suggests the positive effects of the commensal microbiota on the neuropsychiatric and behavioral pathology (Desbonnet et al., 2008; Messaoudi et al., 2011; Bravo et al., 2011). Further, the efficacy of prebiotics in augmenting the growth of microbiota is well established and is likely to afford benefits to the brain. However, the potential of prebiotics to modulate the neurotoxic responses in the developing brain that may arise due to maternal exposure to neurotoxicants has received little attention. Nevertheless, the physiological benefits that accrue with consumption of prebiotic such as NDO during pregnancy is clearly established (van Vlies et al., 2012). It is in this context, the present study, adds a novel observation that maternal IN supplementation does alleviate neurotoxicant-induced oxidative stress and neurotoxicity in maternal as well as fetal brain of rats. We chose ACR as the model neurotoxicant since its developmental neurotoxicity is well demonstrated in experimental

animals (Ferguson et al., 2010; Pedersen et al., 2012). Further, the choice of ACR as the model was also related to the fact that humans are exposed to ACR through diet at very low concentrations chronically owing to the increased consumption of thermally processed carbohydrate-rich foods (Riboldi et al., 2014).

To test our working hypothesis, initially we established basal data on the sublethal doses of ACR and its effects on maternal brain regions and fetal brain in terms of oxidative status, antioxidant defenses, and neurotoxic response. The involvement of generation of oxidative free radicals as well as a host of other factors (Zhang et al., 2011; Prasad and Muralidhara, 2013) have been evidenced in the brain of rodents exposed to ACR. In the present model, we evidenced significant oxidative impairments both in the fetal brain and maternal brain regions as a consequence of ACR exposure. Enhanced markers of oxidative stress and perturbations in several antioxidant enzyme levels suggested that ACR neurotoxicity may be, at least in part, mediated through the production of oxidative stress species. Enhanced ROS generation is likely due to lower efficiency of detoxifying system in the brain of the developing fetus. Similarly, previous studies have suggested increased susceptibility of offspring brain to ACR neurotoxicity after maternal exposure to ACR (Takahashi et al., 2009; Allam et al., 2011). Type-2 alkenes including ACR is known to interact with nerve terminals by affecting neurotransmission thereby disturbing signaling pathways (LoPachin et al., 2004). Exposure to ACR in the present study markedly enhanced the activities of serine hydrolases (AChE and BuChE) in the fetal brain. Altered activity of cholinesterases may be a potential marker indicating that the crucial regulatory processes are being affected during synapse formation, since cholinesterases are thought to be involved in the regulation of cholinergic transmission, neurite growth and cell proliferation in



**Fig. 5.** Effect of maternal IN supplementation (GD 0–19) on the activity levels of acetylcholinesterase (A) and dopamine levels (B) in maternal brain regions and fetal brain in response to acrylamide (ACR, GD 6–19) exposure in drinking water. Results are expressed as mean  $\pm$  SE ( $n = 6$ ). Data analyzed by one-way ANOVA followed by post hoc Tukey test ( $p < 0.05$ ). \*Significant against control; # significant against ACR.



**Fig. 6.** Effect of maternal IN supplementation (GD 0–19) on the activities of mitochondrial NADH–cytochrome c reductase (complexes I–III) (A), and MTT levels (B) in maternal brain regions (cortex/cerebellum) and fetal brain of rats exposed to acrylamide (ACR, GD 6–19) in drinking water. Results are expressed as mean  $\pm$  SE ( $n = 6$ ). Data analyzed by one-way ANOVA followed by post hoc Tukey test ( $p < 0.05$ ). \*Significant against control; # significant against ACR.

**Table 5**

Effect of gestational inulin (IN) supplementation on total cecum weight, *Bifidobacteria* and *Lactobacillus* numbers among dams exposed to acrylamide (ACR).

Group	Cecum weight (g)	<i>Bifidobacteria</i> <sup>†</sup>	<i>Lactobacillus</i> <sup>†</sup>
Control	3.28 $\pm$ 0.03	9.70 $\pm$ 0.10	7.62 $\pm$ 0.02
IN	3.53 $\pm$ 0.07	10.58 $\pm$ 0.06*	7.96 $\pm$ 0.09
ACR	3.0 $\pm$ 0.03	8.73 $\pm$ 0.04*	7.09 $\pm$ 0.03
INU + ACR	3.24 $\pm$ 0.04	9.16 $\pm$ 0.06*	7.10 $\pm$ 0.04

IN (2 g/kg bw/day) supplements were provided during gestation days (GD) 0–19; ACR (200 ppm) was provided in drinking water during GD 6–19.

Values are mean  $\pm$  SE ( $n = 6$ /group). Data analyzed by one-way ANOVA followed by post hoc Tukey test ( $p < 0.05$ ).

<sup>†</sup> Log colony-forming units/g cecal wet content.

development of nervous system (Brimijoin and Koenigsberger, 1999).

Maternal dietary manipulation is considered as a useful practical strategy in determining fetal development and pregnancy outcome (Al-Gubory et al., 2010). In this perspective, maternal dietary intervention with the use of non-digestible oligosaccharides targeting gut–brain axis may represent a novel strategy to achieve neuroprotection. Having obtained evidence of induction of the oxidative dysfunction in the fetal brain by maternal ACR exposure, next we sought to assess if maternal supplementation with a IN during pregnancy has neuroprotective consequences in the fetal and maternal brains. First, we monitored maternal behavior (on GD 14) employing an open field exploration test since ACR exposure causes specific behavioral phenotype. The data revealed that ACR-treated dams displayed reduced frequency of entries/time spent in the central quadrant of an open field arena. Interestingly, ACR dams supplemented with IN exhibited enhanced exploration of the central quadrant, while there was no effect on the total duration of time spent. It is conceivable that the IN-mediated modulation of other neurotransmitter receptors and pathways (or other unidentified processes) within the brain regions which regulate the motivational/or other processes may have contributed to the observed difference across the two tested exploratory domains. Although speculative, enhanced exploration of the center zone by dams may be related to the selective proliferation of *Bifidobacteria* numbers with IN supplementation. We observed no increase in cecal weight following IN supplementation, although it is well demonstrated that oligosaccharide supplementation causes significant increase in cecal weight in animal models. We speculate that this effect may be related to the dose and duration of IN supplements. Further we do not rule out the possibility of IN causing enhanced colonization of other indigenous bacterial strains. Our speculation is supported by the previously reported anxiolytic effect of a probiotic *Lactobacillus rhamnosus* (Lr JB-1) in mice (Bravo et al., 2011). Recently, several researchers have demonstrated a link between enteric microbiota and brain function (Savignac et al., 2013; Heijtz et al., 2011). Although the exact mechanism by which IN-induced altered exploratory behavior is not clear from this study, it can be reasonably speculated to be mediated through the involvement of increased *Bifidobacteria* numbers. This line of thinking is consistent with recent findings which have shown the involvement of the neuroactive substance (GABA) and vagus nerve involvement in non-anxious behavior associated with proliferation of gut microbiota (Bercik et al., 2011). Further, anxiolytic effect could probably stem from altered expression of brain derived neurotrophic factor (BDNF) supporting the proposition that prebiotics increase the availability of trophic support (Savignac et al., 2013). However, further studies are needed to better understand the role of enhanced microbiota in alleviating the neurotoxicant induced adverse phenotype.

In the present study, IN supplementation among ACR dams markedly diminished the levels of ROS and MDA in maternal brain regions and fetal brain coupled with lowering of protein carbonylation in maternal brain regions along with enhanced antioxidant enzyme activities.

These findings are concordant with reports which demonstrated the anti-oxidative potential of IN in vitro (Stoyanova et al., 2011) and the potency of oligofructoses to scavenge ROS in an in vivo model of high fructose diet (Van den Ende et al., 2011). Recent studies have demonstrated similar protective effect of IN against CCl<sub>4</sub> and bacterial endotoxin (LPS) induced toxicities (Liu et al., 2015; Pasqualetti et al., 2014). Further, in the present model, IN supplementation restored the activity levels of thioredoxin reductase among ACR dams and this is important in light of the evidence that impaired selenoproteins likely represent a mediator of developmental brain damage (Soerensen et al., 2008). Additionally, IN supplements significantly prevented the enhanced activity of AChE in the fetal brain suggesting that it may be useful in regulating cholinergic function in the brain. However, further investigations are necessary to obtain insights into the underlying mechanism/s by which IN supplements cause these physiological effects. We speculate that these may be related at least in part to the recently reported capacity of certain microbes to generate neurometabolites (Lyte, 2011) and also to the role of vagus nerve stimulation by gut microbiota and dietary components (Forsythe et al., 2014). These findings also are suggestive of a possible role of IN in the modulation of parasympathetic function. Collectively, these results implicate the protective role of IN against ACR-induced oxidative stress. Consistent with our earlier report showing that ACR exposure has been linked to oxidative damage coupled with mitochondrial dysfunction (Prasad and Muralidhara, 2014), in the present investigation we found that although diminished activity of complexes I–III was observed in fetal brain, IN supplements did not appreciably influence the activity levels in maternal brain.

Several studies have demonstrated that certain probiotic bacterial strains possess significant ability to remove a variety of foodborne mutagens including heterocyclic amines and mycotoxins (Orrhage et al., 1994; Serrano-Nino et al., 2013). In a recent study, the ability of selected *Lactobacillus* strains to bind ACR was demonstrated in vitro suggesting their potential to detoxify this food contaminant (Serrano-Nino et al., 2014). Although these evidences suggest that IN supplements may affect the bioavailability of ACR in vivo, since we did not measure the glycidamide levels in our study, it is rather difficult to conclusively state the effect of IN on ACR metabolism. Further, the possibility of IN interaction with ACR in vivo prior to digestion cannot be totally ruled out. It would be worthwhile to obtain further insights on the precise role of IN in modulating the bioavailability of ACR.

Relatively little is known concerning the role oligosaccharides on the central monoamine level in rodents. Interestingly, although maternal IN supplementation did not influence fetal brain DA level, significant improvement was evident only in the maternal cortex. It is quite likely that IN-mediated enhanced DA levels might influence dopaminergic neuronal activity. While the underlying mechanism/s by which IN modulates DA levels remain unclear from the present study, it correlated well with bacterial count. Our finding corroborates a recent study in which neurotransmitter turnover and amino acid metabolism have been shown to be regulated by intrinsic enteric microbiota (O'Mahony et al., 2014) and the hypothesis on the microbial influence on neural processes within the gastrointestinal tract can affect brain neurotransmission.

Collectively, our findings indicate that IN supplements during gestation possess significant modulatory effect on the maternal phenotype and significantly alleviate the neurotoxicant associated oxidative induction. Further, this has a significant effect on the developing fetal brain as well. However, further studies should aim to understand the possible mechanism/s by which IN can substantially modulate the exploratory behavior and ameliorate ACR neurotoxicity both in the fetus and mother.

## 5. Conclusion

The use of dietary approaches such as prebiotic oligosaccharides during pregnancy to modulate beneficial microbiota that in turn can abrogate the neurotoxic implications during fetal development provides a

novel approach to understanding the interaction between microbiota and brain.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Transparency document

The Transparency document associated with this article can be found, in the online version.

## Acknowledgments

We thank the Director, Central Food Technological Research Institute for his keen interest in this study. The first author was recipient of Junior and Senior Research Fellowships from Council of Scientific and Industrial Research (CSIR), New Delhi. Authors gratefully acknowledge Ms. Divyashri G and Dr. Prapulla SG, Fermentation Technology & Bioengineering Department, CSIR-CFTRI for their kind help in the microbiological procedures.

## References

- Aebi H. Catalase in vitro. *Methods Enzymol* 1984;105:121–6.
- Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Int J Biochem Cell Biol* 2010;42: 1634–50.
- Allam A, El-Ghareeb AA, Abdul-Hamid M, Baikry A, Sabri MI. Prenatal and perinatal acrylamide disrupts the development of cerebellum in rat: Biochemical and morphological studies. *Toxicol Ind Health* 2011;27:291–306.
- Annola K, Keski-Rahkonen P, Vähäkangas K, Lehtonen M. Simultaneous determination of acrylamide, its metabolite glycidamide and antipyrine in human placental perfusion fluid and placental tissue by liquid chromatography-electrospray tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008;876:191–7.
- Bercik P, Park AJ, Sinclair D, Khoshdel A, Lu J, Huang X, et al. The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut–brain communication. *Neurogastroenterol Motil* 2011;23:1132–9.
- Berridge MV, Tan AS. Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction. *Arch Biochem Biophys* 1993;303:474–82.
- Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A* 2011;108: 16050–5.
- Brimijoin S, Koenigsberger C. Cholinesterases in neural development: new findings and toxicologic implications. *Environ Health Perspect* 1999;107:59–64.
- Collins SM, Bercik P. Gut microbiota: intestinal bacteria influence brain activity in healthy humans. *Nat Rev Gastroenterol Hepatol* 2013;10:326–7.
- Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* 2012;13:701–12.
- Cryan JF, O'Mahony SM. The microbiome–gut–brain axis: from bowel to behavior. *Neurogastroenterol Motil* 2011;23:187–92.
- Dalpiaz A, Filosa R, de Capriaris P, Conte G, Bortolotti F, Biondi C, et al. Molecular mechanism involved in the transport of a prodrug dopamine glycosyl conjugate. *Int J Pharm* 2007;336:133–9.
- Desbonnet L, Garrett L, Clarke G, Bienenstock J, Dinan TG. The probiotic *Bifidobacteria infantis*: An assessment of potential antidepressant properties in the rat. *J Psychiatr Res* 2008;43:164–74.
- Ellman GL, Courtney KD, Andres V, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961;7:88–95.
- El-Sayyad HI, Abou-Egla MH, El-Sayyad FI, El-Ghawet HA, Gaur RL, Fernando A, et al. Effects of fried potato chip supplementation on mouse pregnancy and fetal development. *Nutrition* 2011;27:343–50.
- Erkekoglu P, Baydar T. Acrylamide neurotoxicity. *Nutr Neurosci* 2014;17:49–57.
- Ferguson SA, Garey J, Smith ME, Twaddle NC, Doerge DR, Paule MG. Preadolescent behaviors, developmental landmarks, and acrylamide and glycidamide levels after pre- and postnatal acrylamide treatment in rats. *Neurotoxicol Teratol* 2010;32: 373–82.
- Flohe L, Gunzler WA. Assays of glutathione peroxidase. *Methods Enzymol* 1984;105: 114–21.
- Forsythe P, Bienenstock J, Kunze WA. Vagal pathways for microbiome–brain–gut axis communication. *Adv Exp Med Biol* 2014;817:115–33.
- Garey J, Paule MG. Effects of chronic oral acrylamide exposure on incremental repeated acquisition (learning) task performance in Fischer 344 rats. *Neurotoxicol Teratol* 2010;32:220–5.

- Gokul K, Muralidhara. Oral supplements of aqueous extract of tomato seeds alleviate motor abnormality, oxidative impairments and neurotoxicity induced by rotenone in mice: relevance to Parkinson's disease. *Neurochem Res* 2014;39:1382–94.
- Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. *Lancet* 2006;368:2167–78.
- Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. *Lancet Neurol* 2014;13:330–8.
- Guthenberg C, Alin P, Mannervik B. Glutathione transferase from rat testis. *Methods Enzymol* 1985;113:507–10.
- Heijtz RD, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci U S A* 2011;108:3047–52.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives). Evaluation of certain contaminants in food. WHO Technical Report Series, 960. Rome, Italy: JECFA; 2011.
- Kelly G. Inulin-type prebiotics – a review. *Altern Med Rev* 2008;13:315–29.
- Kostyuk VA, Potapovich AI. Superoxide-driven oxidation of quercetin and a simple sensitive assay for determination of superoxide dismutase. *Biochem Int* 1989;19:1117–24.
- Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990;186:464–78.
- Littlejohn D, Mangano E, Shukla N, Hayley S. Interferon-gamma deficiency modifies the motor and co-morbid behavioral pathology and neurochemical changes provoked by the pesticide paraquat. *Neuroscience* 2009;164:1894–906.
- Liu J, Lu JF, Wen XY, Kan J, Jin CH. Antioxidant and protective effect of inulin and catechin grafted inulin against CCl<sub>4</sub>-induced liver injury. *Int J Biol Macromol* 2015;72:1479–84.
- LoPachin RM. The changing view of acrylamide neurotoxicity. *Neurotoxicology* 2004;25:617–30.
- LoPachin RM, Gavin T. Acrylamide-induced nerve terminal damage: relevance to neurotoxic and neurodegenerative mechanisms. *J Agric Food Chem* 2008;56:5994–6003.
- LoPachin RM, Gavin T. Molecular mechanism of acrylamide neurotoxicity: lessons learned from organic chemistry. *Environ Health Perspect* 2012;120:1650–7.
- LoPachin RM, Schwarcz AI, Gaughan CL, Mansukhani S, Das S. In vivo and in vitro effects of acrylamide on synaptosomal neurotransmitter uptake and release. *Neurotoxicology* 2004;25:349–63.
- Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurements using Folin-phenol reagent. *J Biol Chem* 1951;193:265–75.
- Luthman M, Holmgren A. Rat liver thioredoxin and thioredoxin reductase: purification and characterization. *Biochemistry* 1982;21:6628–33.
- Lyte M. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *Bioessays News Rev Mol Cell Dev Biol* 2011;33:574–81.
- Manson J, Brabec MJ, Buelke-Sam J, Carlson GP, Chapin RE, Favor JB, et al. NTP-CERHR expert panel report on the reproductive and developmental toxicity of acrylamide. *Birth Defects Res B Dev Reprod Toxicol* 2005;74:17–113.
- Marques TM, Cryan JF, Shanahan F, Fitzgerald GF, Ross RP, Dinan TG, et al. Gut microbiota modulation and implications for host health: dietary strategies to influence the gut-brain axis. *Innovative Food Sci Emerg Technol* 2014;22:239–47.
- Martyniuk CJ, Feswick A, Fang B, Koomen JM, Barber DS, Gavin T, et al. Protein targets of acrylamide adduct formation in cultured rat dopaminergic cells. *Toxicol Lett* 2013;219:279–87.
- Messaoudi M, Lalonde R, Violette N, Javelot H, Desor D, Nejdi A, et al. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br J Nutr* 2011;105:755–64.
- Mokrasch LC, Teschke Ej. Glutathione content of cultured cells and rodent brain regions: a specific fluorometric assay. *Anal Biochem* 1984;140:506–9.
- Moreadith RW, Fiskum G. Isolation of mitochondria from ascites tumor cells permeabilized with digitonin. *Anal Biochem* 1984;137:360–7.
- Navarro A, Gomez C, Lopez-Cepero JM, Boveris A. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. *Am J Physiol Regul Integr Comp Physiol* 2004;286:R505–11.
- O'Mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav Brain Res* 2014;277:32–48.
- O'Sullivan E, Barrett E, Grenham S, Fitzgerald P, Stanton C, Ross RP, et al. BDNF expression in the hippocampus of maternally separated rats: does *Bifidobacterium breve* 6330 alter BDNF levels? *Benefic Microbes* 2011;2:199–207.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351–8.
- Orrhage K, Sillerstrom E, Gustafsson JA, Nord CE, Rafter J. Binding of mutagenic heterocyclic amines by intestinal and lactic acid bacteria. *Mutat Res Mol Mech Mutagenesis* 1994;311:239–48.
- Pasqualetti V, Altomare A, Guarino MPL, Locato V, Cocca S, Cimini S, et al. Antioxidant activity of inulin and its role in the prevention of human colonic muscle cell impairment induced by lipopolysaccharide mucosal exposure. *PLoS One* 2014;9:e98031.
- Pedersen M, von Stedingk H, Botsivali M, Agramunt S, Alexander J, Brumborg G, et al. Birth weight, head circumference, and prenatal exposure to acrylamide from maternal diet: the European prospective mother-child study (NewGeneris). *Environ Health Perspect* 2012;120:1739–45.
- Prasad SN, Muralidhara. Evidence of acrylamide induced oxidative stress and neurotoxicity in *Drosophila melanogaster* – its amelioration with spice active enrichment: relevance to neuropathy. *Neurotoxicology* 2012;33:1254–64.
- Prasad SN, Muralidhara. Neuroprotective efficacy of eugenol and isoeugenol in acrylamide-induced neuropathy in rats: behavioral and biochemical evidence. *Neurochem Res* 2013;38:330–45.
- Prasad SN, Muralidhara. Mitigation of acrylamide-induced behavioral deficits, oxidative impairments and neurotoxicity by oral supplements of geraniol (a monoterpene) in a rat model. *Chem Biol Interact* 2014;223C:27–37.
- Qiu C, Coughlin KB, Frederick IO, Sorenson TK, Williams MA. Dietary fiber intake in early pregnancy and risk of subsequent preeclampsia. *Am J Hypertens* 2008;21:903–9.
- Riboldi BP, Vinhas AM, Moreira JD. Risks of dietary acrylamide exposure: a systematic review. *Food Chem* 2014;157:310–22.
- Rice D, Barone S. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect* 2000;108:511–33.
- Savignac HM, Corona G, Mills H, Chen L, Spencer JPE, Tzortzis G, et al. Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-D-aspartate receptor subunits and D-serine. *Neurochem Int* 2013;63:756–64.
- Serrano-Nino JC, Cavazos-Garduno A, Hernandez-Mendoza A, et al. Assessment of probiotic strains ability to reduce the bioaccessibility of aflatoxin M1 in artificially contaminated milk using an in vitro digestive model. *Food Control* 2013;31:202–7.
- Serrano-Nino JC, Cavazos-Garduno A, Gonzalez-Cordova AF, Vallejo-Cordoba B, et al. In vitro study of the potential protective role of *Lactobacillus* strains by acrylamide binding. *J Food Saf* 2014;34:62–8.
- Shivananjappa MM, Muralidhara. Abrogation of maternal and fetal oxidative stress in the streptozotocin-induced diabetic rat by dietary supplements of *Tinospora cordifolia*. *Nutrition* 2012;28:581–7.
- Soerensen J, Jakupoglu C, Beck H, Förster H, Schmidt J, Schmahl W, et al. The role of thioredoxin reductases in brain development. *PLoS One* 2008;3:e1813.
- Stoyanova S, Geuns J, Hideg E, Van Den Ende W. The food additives inulin and stevioside counteract oxidative stress. *Int J Food Sci Nutr* 2011;62:207–14.
- Takahashi M, Shibutani M, Nakahigashi J, Sakaguchi N, Inoue K, Morikawa T, et al. Limited lactational transfer of acrylamide to rat offspring on maternal oral administration during the gestation and lactation periods. *Arch Toxicol* 2009;83:785–93.
- Van den Ende W, Peshev D, De Gara L. Disease prevention by natural antioxidants and prebiotics as ROS scavengers in the gastro-intestinal tract. *Trends Food Sci Technol* 2011;22:689–97.
- van Vlies N, Hogenkamp A, Thijssen S, Dingjan GM, Knipping K, Garssen J, et al. Effects of short-chain galacto- and long-chain fructo-oligosaccharides on systemic and local immune status during pregnancy. *J Reprod Immunol* 2012;94:161–8.
- Verma A, Shukla G. Administration of prebiotic inulin suppresses 1,2 dimethylhydrazine dihydrochloride induced procarcinogenic biomarkers fecal enzymes and preneoplastic lesions in early colon carcinogenesis in Sprague Dawley rats. *J Funct Foods* 2013;5:991–6.
- von Stedingk H, Wikstrom AC, Rydberg P, Pedersen M, Nielsen JKS, Segerbäck D, et al. Analysis of hemoglobin adducts from acrylamide, glyciamide, and ethylene oxide in paired mother/cord blood samples from Denmark. *Chem Res Toxicol* 2011;24:1957–65.
- Wolff SP. Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods Enzymol* 1994;233:182–9.
- Yasuda K, Maiorano R, Welch RM, Miller DD, Lei XG. Cecum is the major degradation site of ingested inulin in young pigs. *J Nutr* 2007;137:2399–404.
- Zhang L, Gavin T, Barber DS, LoPachin RM. Role of the Nrf2-ARE pathway in acrylamide neurotoxicity. *Toxicol Lett* 2011;205:1–7.