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Oral Supplementation of GABA Containing Rice Flour Alleviate Acrylamide Induced Oxidative Impairments and Neurotoxicity in Mice

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Abstract

Endogenous antioxidant cycle play a pivotal role in the prevention of damage induced by free radicals. An imbalance between the production and removal of free radicals and reactive oxygen species (ROS) enhances the rate of oxidative stress. Oxidative stress play a major role in the development of neurodegerative diseases. Antioxidants are now being looked as a therapeutic agents, as they have ability to neutralize free radicals. Rice, being the staple food for nearly 50% of world's population, play a vital role in the concentration of antioxidants ingested daily. The present study was designed to investigate antioxidative and neuroprotective effect of gamma-aminobutyric acid (GABA) containing fermented rice flour (GFRF) in acrylamide (ACR) acrylamide (ACR) induced oxidative stress mice model. GFRF contained 750.55 ± 26.03 mg GABA/100g DM under optimized conditions and fermentation enhanced the levels of antioxidant phenolics in GFRF (unpublished data). ACR administration caused significant oxidative stress as evident by increased ROS, protein carbonyls, nitric oxide and malondialdehyde levels in cortex and cerebellum clearly suggesting significant oxidative stress in vivo. Mice supplemented with GFRF orally, diminished oxidative markers, increased the activities of antioxidant enzymes in cortex and cerebellum brain region with concomitant increase in GABA levels, conferring neuroprotection against ACR induced oxidative stress. Our data suggest that antioxidative and neuroprotective effects exerted by GFRF are mediated through decreases in free-radical generation and increases in activities of superoxide dismutase (SOD) and catalase (CAT) *in vivo*.

Keywords: *Antioxidants; Reactive Oxygen Species; Gamma-Aminobutyric Acid; Neuroprotection; GABA Containing Fermented Rice Flour*

Introduction

Oxidative stress and lipid peroxidation are believed to play a significant role in the development neurodegenerative diseases. An imbalance between the production and removal of free radicals and reactive oxygen species (ROS) increases the rate of oxidative stress [1]. The delicate balance between the production and catabolism of oxidants is critical for maintenance of the biological function [2]. Exposure to chemicals that are widely disseminated in the environment and occupational surroundings can cause deleterious effects to the nervous system [3]. Acrylamide (ACR) is a well-recognized neurotoxin that has multiple chemical and industrial applications [4]. Its wide spread application is associated with pollution and health risks [5]. Furthermore, ACR is also a common contaminant in foods prepared

by cooking at high temperatures [6]. Direct consumer exposure to ACR may result from ingestion of high-carbohydrate foods and indirect exposure may result from residual traces of the ACR monomer in food packaging where polyacrylamide is used as a binding agent [7]. Several hypotheses have been suggested to explain the molecular mechanisms of neurotoxic action of the ACR: enhancement in the rate of ROS production and carbonyl content, formation of adducts with reduced glutathione (GSH), interaction with nucleic acids, second messenger systems, enzymes, receptors or translocating proteins, reduction of enzymatic and non-enzymatic antioxidants with decrease in acetylcholine esterase (AChE) activity, effects on neurotransmitter concentrations and reuptakes, disruption of membrane dynamics, and damage to glial cells resulting in accelerated lipid peroxidation that ultimately influences neuronal function [6,8]. The electrophilic nature of ACR suggests that this neurotoxicant adds nucleophilic sulfhydryl groups on cellular proteins that are critically involved in membrane fusion [4].

Rice, being the main food in the diet of most populations, play an important role in the concentration of antioxidants ingested daily [9]. Scientific evidence have demonstrated a positive correlation between the concentration of phenolic compounds in rice and its antioxidant activity [10,11]. Therefore, the suppression of oxidative stress by natural antioxidant compounds seems to be an effective approach in preventing the initiation and progression of disease [12].

Lactic acid fermentation is a vital method to exploit the functional potential of rice and to enrich them with bioactive compounds [13]. Indeed, the lactic acid fermentation has widely been used to enhance the antimicrobial, antioxidant and immune-modulatory features of several cereal, pseudo-cereal and leguminous flours as well as medicinal plants like *Echinacea* spp. [14]. In addition, lactic acid bacteria (LAB) has been used for GABA synthesis from grape must [15], to enrich GABA in sourdough fermentation (Coda., *et al*. 2010), to enhance antioxidant and antihypertensive peptides from various cereal and pseudo-cereal flours [16]. In our study, lactic acid fermentation of rice flour was carried out to enhance the levels of antioxidants and GABA in the fermented rice flour. GABA is a non-protein amino acids that functions as a major inhibitory neurotransmitter in mammalian central nervous system (CNS). Numerous studies have stated the physiological function of GABA viz., as an antihypertension, anticancer, anti-inflammatory and hepatoprotective agent [1-19]. These aspects have encouraged the interest to formulate GABA-containing natural product [19].

To date, no *in vivo* test has been conducted to assess the neuroprotective potential of GABA containing fermented rice (GFRF). Thus, the purpose of the study was to evaluate antioxidant and neuroprotective effect of GFRF under the conditions of ACR induced oxidative stress and neurotoxicity.

Materials and Methods

Bacterial strain and growth conditions

Enterococcus faecium NCIM 5593 used in the study was isolated from indigenous fermented food and identified using genomic techniques [20]. The strain has earlier been reported to be a potent GABA producer [21] and the laboratory GABA production process is optimized and the strain has shown its potential to produce GABA under simulated gastro-intestinal conditions [20,22]. The strain was maintained at -80°C in de Man Rogosa and Sharpe (MRS) broth with 10% (v/v) glycerol and was normally cultured in MRS broth at 37°C for regular experiments.

Preparation of GABA containing fermented rice flour (GFRF)

GABA containing fermented rice flour was prepared as described in our previous publication [23]. Briefly, rice flour (3% w/v) supplemented with glutamate (1% w/v) was sterilized at 121°C for 20 mins, inoculated with *E. faecium* NCIM 5593 and incubated at 37°C, 120 rpm for 60h of fermentation time (optimized conditions). The resulting fermented slurry was thermally treated at 70°C for 10 mins, centrifuged at 8000 rpm for 20 mins and the resulting water extract was lyophilized at -80°C to yield GFRF extract. GABA in the extract

was analyzed using HPLC as described in our earlier publication [20]. At the end of fermentation, 750.55 ± 26.03 mg GABA/100g DM was determined. GABA content in control RF was found to be 0.86 ± 0.02 mg/100 DM.

Animals and Diet

Male mice (CFT-Swiss; 6 weeks old, weighing 20 - 25 g) obtained from CSIR-CFTRI animal facility were housed three per cage in standard polypropylene cages (27x21x14-cm) provided under standard experimental conditions: RT 23 ± 2°C, humidity 40 - 60%, 12-h light/dark cycle. Mice were acclimatized for one week prior to the start of the experiment and were maintained on commercial diet and tap water *ad libitum*. All experiments were conducted strictly in accordance with approved guidelines by the 'Institutional Animal Ethics Committee' (IAEC) regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division, Government of India, India. Mice were weighed and randomly assigned to ten groups of 6 animals each before the commencement of experiment.

Dose and grouping

Control (CTR) group mice received saline. RF treated mice groups (RF1 and RF2 groups) received 500 and 1000 mg RF extract/kg bw (p.o. daily) respectively. GFRF treated mice groups (GFRF1 and GFRF2) received 500 and 1000 mg GFRF extract/kg bw (p.o. daily) respectively. 500 and 1000 mg GFRF extract/kg bw corresponds to 2.6 mg and 5.2 mg GABA/kg bw, respectively. A10, A10+RF1, A10+RF2, A10+GFRF1 and A10+GFRF2 group mice were administered ACR at a dose of 10 mg/kg bw (3 times a week). A10+RF1 and A10+RF2 group mice were supplemented with 500 and 1000 mg RF extract/kg bw (p.o. daily), respectively. A10+GFRF1 and A10+GFRF2 group mice were supplemented with 500 and 1000 mg GFRF extract/kg bw (p.o. daily) respectively. RF and GFRF extracts were supplemented one hour prior to ACR administration. Weekly body weights were recorded throughout the experimental period (4 weeks). At the end of the study, mice of all the groups were subjected to behavioral tests between 09:00 and 14:00h. Finally, mice of all the group were sacrificed under light anesthesia and brain was excised and brain regions (cortex, Ct and cerebellum, Cb) were dissected. All the brain regions were stored at -80°C until further processing for isolation of the cytosol and subsequently for biochemical estimations and neurochemical quantification.

Behavioral analysis: Open field test and Elevated plus maze

Assessment of exploratory activity and anxiety-like behavior of mice were evaluated using open field test and elevated plus maze as described in our previous study [24].

Sample preparation and biochemical assays

Cytosolic fractions from brain regions (Ct and Cb) was isolated with minor modifications [25]. Homogenate (10% w/v) of the brain regions was prepared in ice-cold homogenizing Tris-Sucrose buffer (100 mM sucrose, 10 mM EDTA, 100 mM Tris-HCl; pH 7.4) using a glass-Teflon grinder. Resulting homogenate was centrifuged at 3,000 rpm for 10 min at 4°C. Cytosolic fractions thus obtained by centrifugation at 10,000 rpm for 10 min at 4°C was stored at -80°C until further use.

Oxidative markers

Reactive oxygen species (ROS) and protein carbonyls (PC) were assayed as described in our previous publication [24]. ROS formation was quantified from a standard 2',7'-dichlorofluorescein (DCF) standard curve and the results were expressed as pmol DCF formed/min/mg protein. PC were expressed as nmoles carbonyls/mg protein. NO levels were measured by using commercially available Griess reagent and were quantified using sodium nitrate standard curve [26]. GSH levels were quantified by fluorescence as described by Mokrasch and Teschke [27]. GSH concentration was calculated from the standard curve and the results were expressed as µg GSH/mg protein. Lipid peroxidation was assessed by measuring free malondialdehyde (MDA) as described by Ohkawa., *et al.* [28].

Enzyme activities: Catalase (CAT) and Superoxide Dismutase (SOD)

CAT activity was measured as described previously [24] and the activity was expressed as nmol H2O2 hydrolyzed/min/mg protein (ε-44.2/mM/cm). SOD activity was assayed by monitoring the inhibition of QE auto-oxidation [29].

Acetylcholinesterase (AChE) activity

AChE activity was determined as described earlier [24]. The enzyme activity was expressed as nmoles substrate hydrolyzed/min/mg protein.

GABA levels

GABA levels were quantified using HPLC [22].

Protein estimation

Protein concentration in the samples was determined by Lowry's method using bovine serum albumin as standard [30].

Statistical Analysis

The experimental data obtained were expressed as mean ± SE. Statistical analysis was performed using InStat3 software (v3.36). The differences were analyzed by one-way ANOVA followed by a post-hoc Tukey's test.

Results and Discussion

Body weight

Neuroprotective efficacy of GFRF in attenuating oxidative stress and neurotoxicity in mice exposed to ACR was validated. Body weight of mice fed with oral supplementation of RF and GFRF at both doses was not significantly different when compared to CTR group mice (Table 1). Body weight exhibited a progressive decrease among A10 group mice, while ACR mice fed with RF and GFRF showed significant improvement ($p < 0.05$) in body weight in comparison to A10 group mice (A10+RF1: 22.70%; A10+RF2: 24.93%; A10+GFRF1: 27.27%; A10+GFRF2: 30.16%).

Treatment group	Body weight (g)				
	Initial	Week 1	Week 2	Week 3	Week 4
CTR	30.23 ± 0.94	32.97 ± 1.16	34.92 ± 0.80	36.02 ± 1.03	37.15 ± 0.50
RF1	29.45 ± 0.88	31.78 ± 0.95	33.61 ± 0.81	34.93 ± 0.24	35.45 ± 0.24
RF2	29.77 ± 0.51	32.06 ± 0.53	33.75 ± 0.29	34.87 ± 0.37	36.47 ± 0.24
GFRF1	30.55 ± 0.91	33.17 ± 0.67	34.12 ± 0.77	35.83 ± 1.49	36.05 ± 0.46
GFRF2	29.87 ± 0.33	32.81 ± 0.79	34.87 ± 0.53	35.57 ± 1.19	36.37 ± 0.55
A10	30.15 ± 0.79	31.76 ± 1.21	**29.97 ± 0.31[a]**	**28.75 ± 1.71[a]**	**27.75 ± 0.17[a]**
A10+RF1	30.75 ± 0.86	31.85 ± 0.46	33.97 ± 0.24	33.67 ± 0.31	**34.05 ± 0.16[b]**
A10+RF2	30.27 ± 0.45	32.08 ± 0.94	34.57 ± 0.24	33.27 ± 0.56	**34.67 ± 0.23[b]**
A10+GFRF1	30.53 ± 0.59	32.18 ± 1.11	34.45 ± 0.62	32.23 ± 0.19	**35.32 ± 0.63[b]**
A10+GFRF2	30.95 ± 1.03	32.42 ± 1.07	35.17 ± 0.95	**35.17 ± 0.98[b]**	**36.12 ± 0.34[b]**

Table 1: *Effect of GFRF supplementation on body weight of mice.*

Behavioral analysis: Open field test and Elevated plus maze

ACR administration caused significant increase in anxiety-like behavior as evident by open field test and elevated plus maze. Effect of RF and GFRF on exploratory behavior and elevated plus maze activity was evaluated (Figure 1a and b). Frequency of entries to the center arena in open field test was significantly ($p < 0.05$) reduced in ACR administered mice. ACR mice showed significant ($p < 0.05$) improvement by GFRF supplementation. Likewise, in terms of time spent exploring the center of the open field, ACR mice spent significantly less time, while those receiving GFRF supplements spent relatively more time (Figure 1a). In elevated plus maze, mice given GFRF supplements spent significant ($p < 0.05$) time exploring open arms of maze (Figure 1b). Reduced time spent by ACR administered mice was significantly increased ($p < 0.05$) by GFRF supplementation.

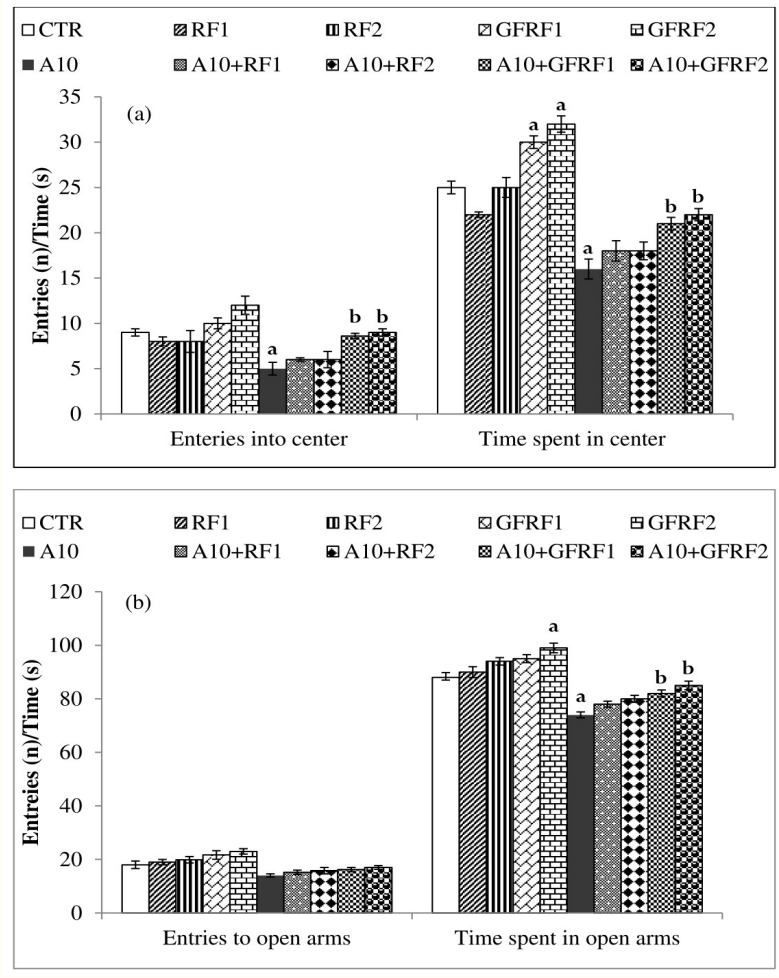

Figure 1: *Effect of GFRF supplementation on exploratory behavior of mice. (a) Open field test (b) Elevated plus maze. The values are mean ± SE. Statistical analysis was performed using one way ANOVA followed by post-hoc Tukey's test. 'a' and 'b' represents statistically significant against control and ACR treated mice.*

Oxidative markers

ACR administration caused significant oxidative stress as evident by increased ROS, PC, NO and MDA levels in Ct and Cb clearly suggesting significant oxidative stress *in vivo*. RF supplementation per se did not significantly affect the cytosolic oxidative markers viz., ROS, PC, NO, MDA and GSH at both doses. Higher dose of GFRF significantly ($p < 0.05$) diminished the levels of ROS in Ct (22.15%) whereas no change was evident in other brain region under normal conditions. ACR administered mice exhibited a significant ($p < 0.05$) increase in ROS levels in Ct (59.42%) and Cb (27.34%) regions of brain (Figure 2). While the elevated levels of ROS were normalized significantly by the supplementation of low (Ct: 48.44% and Cb: 26.06%) and high dose of GFRF (Ct: 66.37% and Cb: 26.28%).

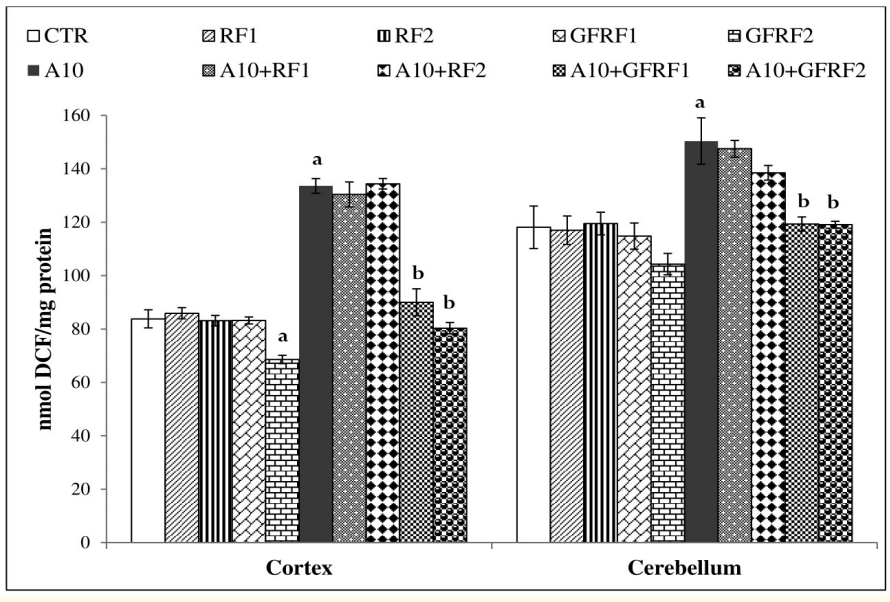

Figure 2: *Effect of GFRF supplementation on ROS levels. The values are mean ± SE. Statistical analysis was performed using one way ANOVA followed by post-hoc Tukey's test. 'a' and 'b' represents statistically significant against control and ACR treated mice.*

Higher dose of GFRF significantly ($p < 0.05$) diminished PC levels only in Ct (25.58%). ACR induced increase in PC levels in Ct (35.74%) were reduced by the supplementation of RF (A10+RF2: 18.60%) and GFRF (A10+GFRF1: 27.03% and A10+GFRF2: 26.59%). Significant increase in PC levels in Cb (20.39%) was observed in ACR administered mice (Figure 3). ACR induced elevation in Cb was significantly ($p < 0.05$) restored by the supplementation of higher dose of RF (15.64%) and GFRF (29.24%).

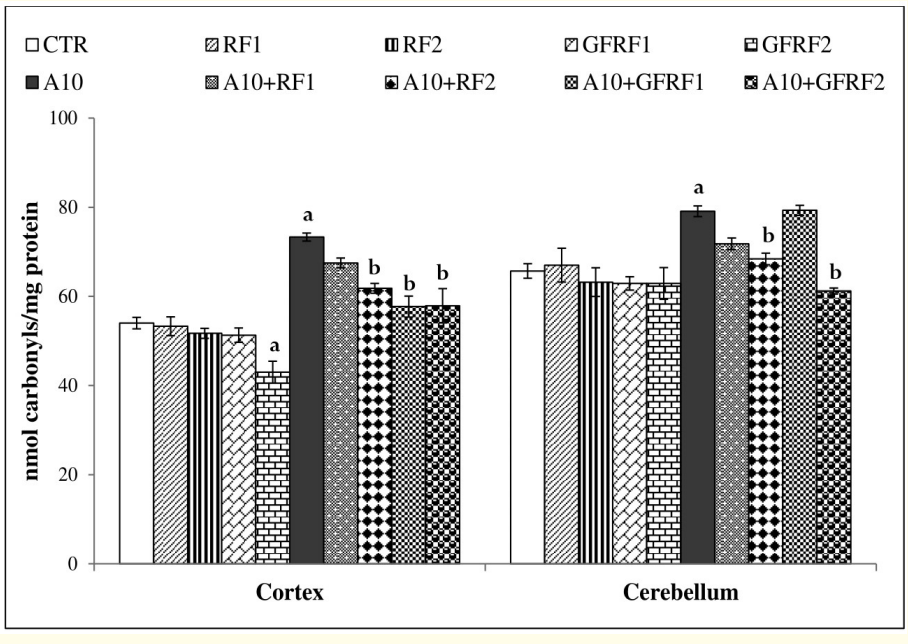

Figure 3: *Effect of GFRF supplementation on PC levels. The values are mean ± SE. Statistical analysis was performed using one way ANOVA followed by post-hoc Tukey's test. 'a' and 'b' represents statistically significant against control and ACR treated mice.*

Furthermore, ACR induced elevation in NO levels in Ct (34.68%) and Cb (22.54%), was normalized in both Ct (34.95%) and Cb (25.82%) among mice supplemented with higher dose of GFRF (Figure 4). Higher dose of GFRF supplementation per se caused significant ($p < 0.05$) reduction in MDA levels in both Ct (23.07%) and Cb (52.17%). Significant increase in MDA levels (Ct: 155.56%, Cb: 56.52%)

evident among ACR exposed mice were significantly ($p < 0.05$) restored in Ct (A10+RF1: 27.08%, A10+RF2: 35.41%, A10+GFRF1 and A10+GFRF2: 72.91%) and Cb (A10+GFRF2: 33.34%) with RF and GFRF supplementation (Figure 5). Supplementation of high dose of GFRF caused significant ($p < 0.05$) enhancement in GSH levels in Ct (28.28%) and Cb (29.70%). Significant depletion (Ct: 38.38%; Cb: 31.38%) in GSH levels was evident among ACR administered mice (Figure 6). While in Cb, higher dose of GFRF exhibited significant increase in GSH levels (31.09%).

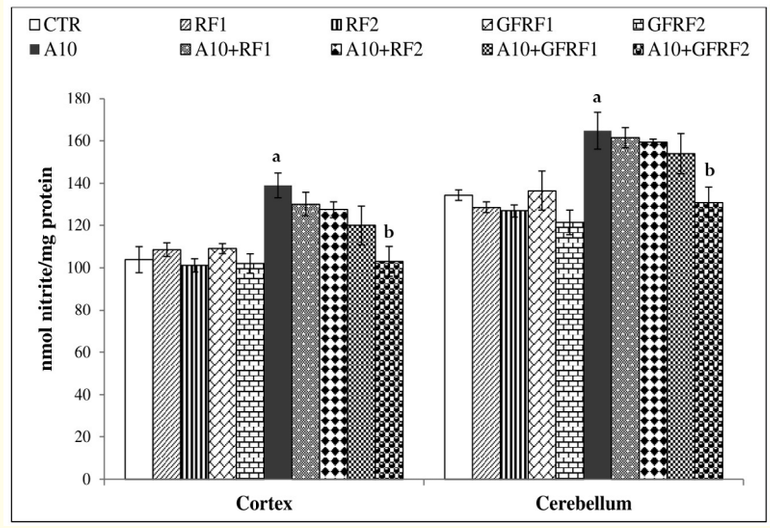

Figure 4: *Effect of GFRF supplementation on NO levels. The values are mean ± SE. Statistical analysis was performed using one way ANOVA followed by post-hoc Tukey's test. 'a' and 'b' represents statistically significant against control and ACR treated mice.*

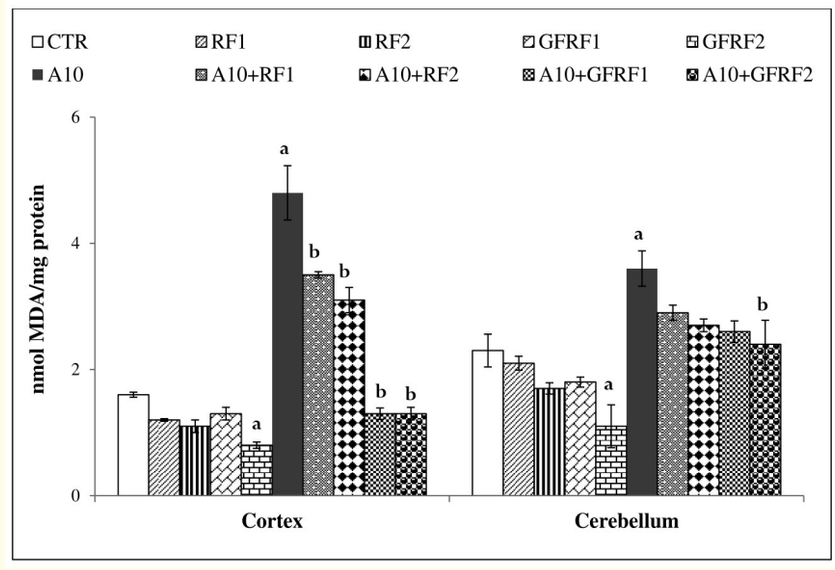

Figure 5: *Effect of GFRF supplementation on MDA levels. The values are mean ± SE. Statistical analysis was performed using one way ANOVA followed by post-hoc Tukey's test. 'a' and 'b' represents statistically significant against control and ACR treated mice.*

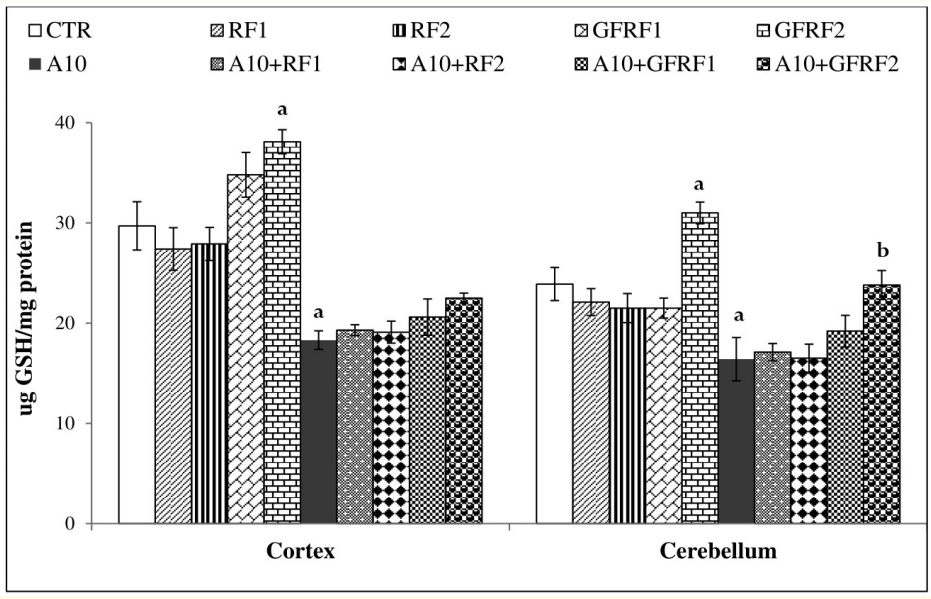

Figure 6: *Effect of GFRF supplementation on GSH levels. The values are mean ± SE. Statistical analysis was performed using one way ANOVA followed by post-hoc Tukey's test. 'a' and 'b' represents statistically significant against control and ACR treated mice.*

Enzyme activities

Supplementation of higher dose of GFRF per se significantly ($p < 0.05$) enhanced the activity of SOD in both Ct (15.38%) and Cb (16.09%). However, low dose of GFRF supplementation could enhance SOD activity significantly ($p < 0.05$) only in Cb (14.33%) (Figure 7a). ACR exposure caused a significant ($p < 0.05$) reduction in SOD activity in the Ct (15.49%) and Cb (25.24%). High dose of GFRF supplementation normalized SOD activity levels both in Ct (18.09%) and Cb (16.70%). Likewise, high dose of GFRF significantly ($p < 0.05$) increased CAT activity in Ct (33.34%) and Cb (54.83%). ACR induced reduction in the activity of CAT in Ct (46.15%) and Cb (57.14%) was significantly ($p < 0.05$) enhanced by GFRF administration (Ct; A10+GFRF2: 61.90%, Cb; A10+GFRF1: 57.14%, A10+GFRF2: 62.50%) (Figure 7b).

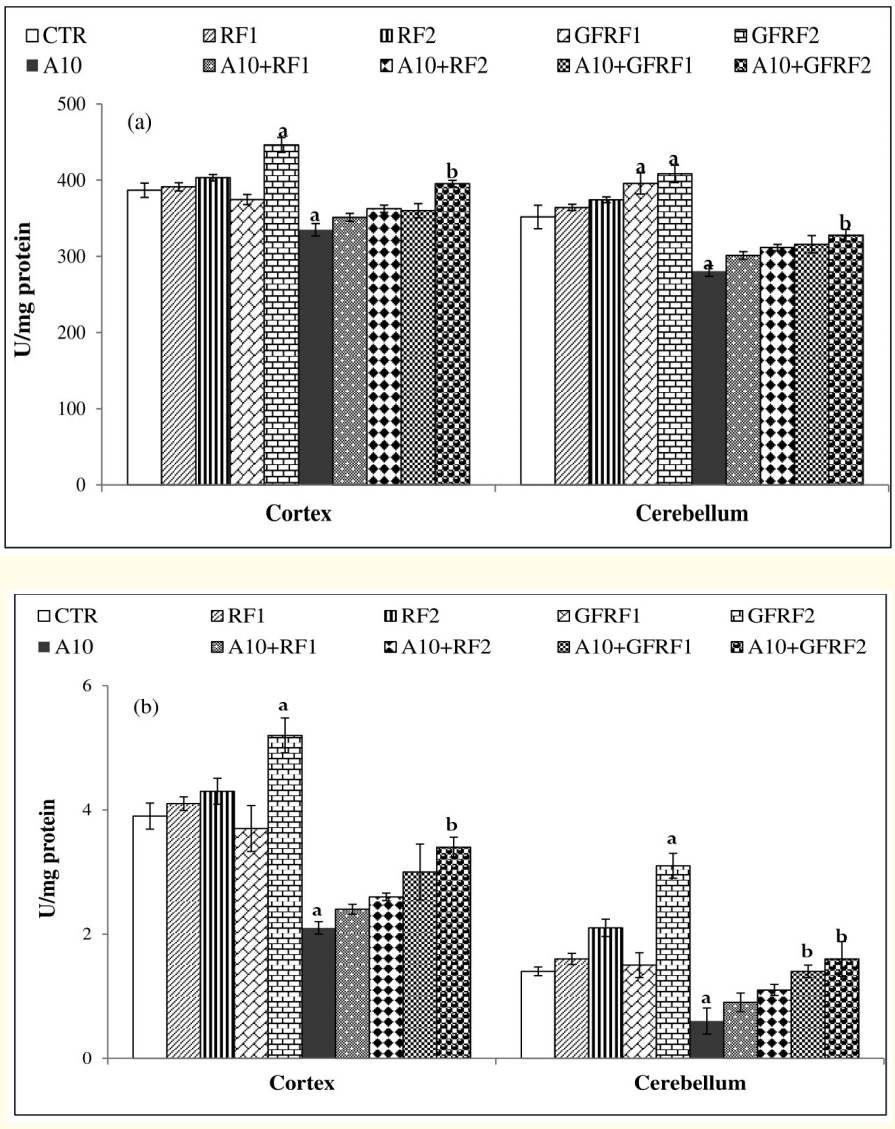

Figure 7: *Effect of GFRF supplementation on antioxidant enzymes activity. (a) SOD (b) CAT. The values are mean ± SE. Statistical analysis was performed using one way ANOVA followed by post-hoc Tukey's test. 'a' and 'b' represents statistically significant against control and ACR treated mice.*

AChE activity

RF and GFRF supplementation per se to mice resulted in differential effect on the AChE activity in brain regions (Figure 8). Activity remained significantly unchanged in Ct and Cb regions. Among ACR exposed dams, AChE activity was markedly enhanced in the cerebellum (66.17%), and cortex (48.02%) and GFRF supplementation restored the activity levels in both the regions (Ct; A10+GFRF2: 35.89%, Cb; A10+GFRF1: 17.67%, A10+GFRF2: 24.69%). However, higher dose of RF could restore ACR diminished activity of AChE only in Cb (17.31%).

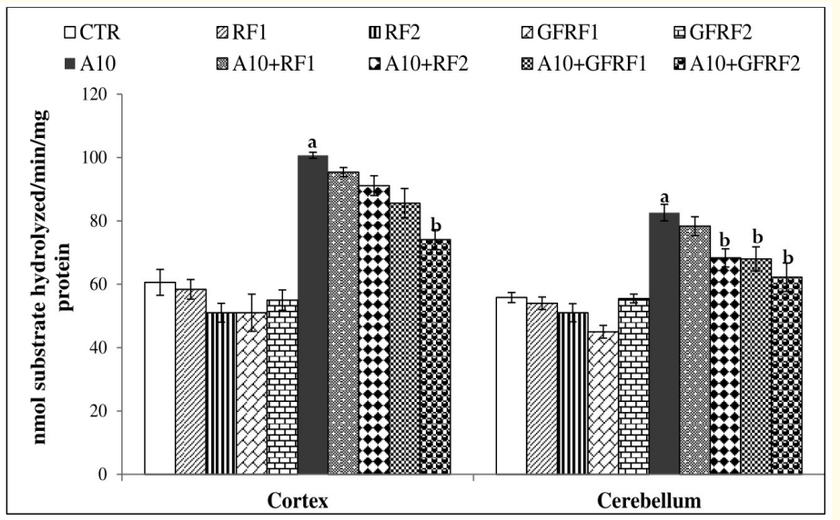

Figure 8: *Effect of GFRF supplementation on AChE level. The values are mean ± SE. Statistical analysis was performed using one way ANOVA followed by post-hoc Tukey's test. 'a' and 'b' represents statistically significant against control and ACR treated mice.*

GABA levels

In general, brain regions of mice given supplements of GFRF showed enhanced cytosolic levels of GABA (Ct; GFRF2: 38.84%, Cb; GFRF1: 25%, GFRF2: 27.27%) (Figure 9). Interestingly, GABA levels were decreased (Ct: 41.67%; Cb: 31.25%) among ACR exposed mice, and RF supplements showed no effect. In contrast, GABA levels that were significantly ($p < 0.05$) depleted in the Ct and Cb among ACR exposed mice, were restored by the supplementation of low (Ct: 30.12% and Cb: 31.25%) and high dose of GFRF (Ct: 50.02% and Cb: 63.63%).

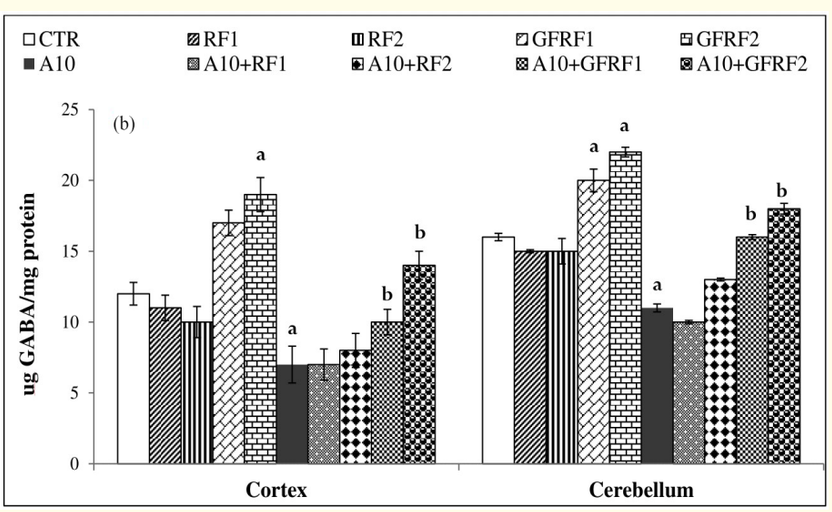

Figure 9: *Effect of GFRF supplementation on GABA level. The values are mean ± SE. Statistical analysis was performed using one way ANOVA followed by post-hoc Tukey's test. 'a' and 'b' represents statistically significant against control and ACR treated mice.*

Discussion

LAB are industrially important microbes that used world over for the preparation of fermented foods. Consumption of lactic acid fermented foods is common worldwide. Fermented foods offer an array of health benefits. In addition to improving its organoleptic quality, fermentation results in improved digestibility and nutritive value [31]. Health benefits gained from the ingestion of fermented plant materials are usually direct, through interaction of ingested live microorganisms with the host (probiotic effect), or indirect as a consequence of ingestion of microbial metabolites, which are synthesized during fermentation (biogenic effect) [32]. Cereal fermentation leads to improvement in product shelf life, nutritional value, and digestibility and significantly lowers antinutritional contents in the final products [33]. Bioactive peptides and GABA may be released during food fermentation at levels higher than the physiological threshold, thus exerting *in vivo* health benefits [32]. Accordingly, present study was aimed to evaluate the efficacy of formulated GABA containing fermented rice flour (GFRF) in mice model. The reason to enhance GABA levels by microbial fermentation is because of its varied health benefits: GABA promotes fat loss by the stimulation of human growth hormone production, increases sleep cycle by giving deeper rest, boosts immune system, lowers blood pressure, inhibits development of cancer cells and also assists in the treatment of anxiety disorders [34,35]. In addition, Ito and Ishikawa [35] reported that GABA has preventive effects on Alzheimer's disease and other cerebral related disorders, such as amnesia and dementia.

Phenolic compounds in rice (exhibit prominent antioxidant properties [36]. The antioxidant phytochemicals in rice are receiving improved consideration for their possible role in prevention of diseases as well as in food quality improvement [37]. Fermentation enhanced the levels of antioxidant phenolics as analyzed by HPLC (unpublished data). It significantly enhanced the levels of antioxidant phenolics in GFRF viz., ferulic acid (78.88%), protocatechuic acid (49.74%), p-coumaric acid (48.31%), syringic acid (46.55%), gallic acid (38.86%), cinnamic acid (13.66%) and sinapic acid (3.28%) in comparison to control (data not shown). Furthermore, short chain fatty acids (SCFAs) were also produced as a result of carbohydrate fermentation in GFRF. Lactic acid (363.12 ± 4.15 mmoles/100 g DM) was found to the major organic acid produced followed by butyric, formic, acetic propionic acid (data not shown).

Neuroprotective efficacy of GFRF in attenuating oxidative stress and neurotoxicity in mice exposed to ACR was validated. Supplementation of GFRF to ACR administered mice, significantly improved mice behavior. Weight loss (7.96%) was observed in ACR treated groups but supplementation of RF and GFRF could significantly inhibit weight loss. ACR administration caused significant increase in anxiety-like behavior as evident by open field test and elevated plus maze. ACR administration caused significant oxidative stress as evident by increased ROS, PC, NO and MDA levels in Ct and Cb clearly suggesting significant oxidative stress *in vivo*. It also caused significant depletion in GSH, SOD and CAT activities. These findings are in line with Shinomol., *et al*. [4] and Prasad and Muralidhara [38].

ROS production increases under pathological conditions, surpassing body's detoxification capacity and results in oxidative stress. Lipid peroxidation produces aldehydes that exacerbate oxidative damage by interacting with nucleic acids and proteins. MDA is the main and most studied product of polyunsaturated fatty acid peroxidation [39]. Reduction in the elevated levels of ROS and MDA upon GFRF supplementation is attributable to their free radical scavenging properties. Protein carbonylation is a type of protein oxidation that is promoted by ROS [40]. Elevated levels of PC are considered as sensitive index of protein oxidation and have been implicated in various neurodegenerative diseases [41]. Elevated levels of PC in Ct and Cb regions among ACR administered mice were effectively brought down by GFRF supplementation. GSH provides the cell with multiple defenses by participating with detoxification process and effectively scavenge free radicals [4]. GFRF per se significantly enhanced GSH levels in Ct and Cb. Interestingly, GFRF offered protection against ACR induced GSH depletion in only Cb. Furthermore, supplementation of GFRF to ACR administered mice could enhance the activities of antioxidant enzymes. An increase in AChE activity has been found to inhibit cell proliferation and promote apoptosis. ACR induced elevation in the AChE activity was significantly restored by the supplementation of GFRF. The studies pertaining to the neuroprotective effect of rice or fermented rice are scarce. Neuroprotective and antioxidative effect of germinated brown rice enriched with GABA and purple rice extract in human SH-SY5Y cells are worth noting [42,43]. ACR caused substantial decrease in GABA levels in both brain regions. Nonethe-

less, supplementation of GFRF could enhance GABA levels significantly. Conversely, no improvement in GABA levels were observed in RF supplemented mice. This strongly supports the fact that observed beneficial effect is due to enhanced GABA concentration and increased levels of antioxidant phenolics in GFRF.

Conclusion

Lactic acid fermentation of rice using *E. faecium* NCIM 5593 could effectively confer neuroprotection against ACR treated mice. The possible mechanism by which GFRF might have influenced brain may be summarized as follows: (1) Vagal nerve activation [44] (2) GABA, ferulic acid and other phenolics in GFRF (3) SCFAs mediated restoration of blood-brain barrier integrity [45]. Hence, based on our findings we can conclude that supplementation of GFRF confers protection against ACR induced oxidative stress.

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