ORIGINAL ARTICLE

Japanese Sake Yeast Potentially Attenuates Arsenic Neurotoxicity in Male Rats Model: Behavioral, Oxidative Stress and Immunogenetics Assessment

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Abstract

Background: Arsenic (AS) is widely distributed in our surroundings, causing various health problems like neurological disorders. The current research was designed to investigate the effect of the anti-oxidant and anti-inflammatory content of sake yeast on the recovery of brain damage in an AS-treated rat's model with behavioral, oxidative stress, and immunogenetics assessment.
Method: Twenty-four male rats were treated with AS (3 mg/kg b.wt. per day) alone or in combination form with sake (45 mg/kg b.wt. per day), and animals received them for 30 days in drinking water (n=6/group). The initial mechanism of action was explored by behavioral tests (rotarod, amphetamine rotation, and spatial memory), oxidative assay, and histopathology methods.
Results: Considering the vehicle group, induction of brain abnormalities by AS significantly ($P<0.05$) decreased the number of substantia nigra neurons, total antioxidant capacity, glutathione peroxidase activity and increased the amount of α-synuclein protein and led to the massive accumulation of malondialdehyde. Meanwhile, sake supplementation can rescue the brain damage caused by this toxic metal, resulting in a reduction of malondialdehyde and α-synuclein protein levels, plus a considerable improvement in blood serum total antioxidant capacity consideration ($P<0.05$). Activity behavioral tests confirmed the AS-mentioned changes by increasing the number of rotations and rod test time. Histopathology assays mimic the above data.
Conclusion: In sum, the sake yeast supplement due to its properties positively influences for improvement of dopaminergic neuron dysfunction via AS damage.

Keywords: α-synuclein, Arsenic, Brain, Oxidative stress, Sake

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INTRODUCTION

In the 21st century world, the general population is exposed to arsenic (AS) toxicity via water, soil, and air in methylated AS species through mining waste, industries using wood preservatives, semiconductors, glass, pesticides, and herbicides [1, 2]. Some results have proved that the brain and nervous system are known as the acute target tissues of chronic AS toxicity [3, 4].

In Iran, the common therapeutic methods for the modulation of AS toxicity in the nervous system often result in unsatisfactory treatment. Therefore, to reduce the damage of AS toxicity on the nervous system of patients, we hope to use some natural substances with healthcare functions to reduce the AS damage and maintain the normal function of the brain. Hence, our local researchers have paid more attention to sake or a traditional Japanese alcoholic beverage supplement, as a novel agent, and its positive globalized effects. The development of new sake yeast strains has been promoted around the world. Modern research has found that sake yeast has anti-oxidant and anti-inflammatory properties for improving the treatment of diabetes [5]. Vaghari et al. [6] reported that the administration of sake could decrease inflammation, and oxidant in a rat model of global cerebral ischemia/reperfusion. Bozorgi et al. [7] indicated one of the newest neurological effects of oral sake yeast supplement exerts a neurobehavioral protective effect predominantly.

The current reseach was designed to investigate the effect

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of the anti-oxidant and anti-inflammatory content of sake yeast on the recovery of brain damage in an AS-treated rat's model with behavioral, oxidative stress, and immunogenetics assessment.

METHODS

Reagents and kits

All reagents were purchased from Sigma-Aldrich (Glasgow, UK) unless otherwise indicated. The kits to evaluate oxidative stress indices were purchased from were purchased from Cosmo Bio Co. (Japan).

Study design

Male adult Sprague Dawley rats (weighing 175±15 g and 8-week-old) obtained from the Pasteur Research Center (Karaj, Iran) were housed in a temperature- and light-controlled conditions. Twenty-four adult male Wistar rats were randomly allocated into four groups (n=6 rats). Group 1 served as normal control group received normal saline, group 2 daily administered AS (3 mg/kg b.wt. per day) dissolved in water by oral gavage [8], group 3 orally gavages by sake (45 mg/kg b.wt. per day as a food supplement) dissolved in water [5], and group 4 was co-administered with AS (3 mg/kg b.wt. per day) and sake (45 mg/kg b.wt. per day).

The study protocol was carried out in compliance with the guidelines for the maintenance and handling of laboratory animals and techniques approved by the Ethics Review Committee of Kermanshah University of Medical Sciences (10-E-IR-KUMS.REC.10-B-1401).

Behavioral tests

Rats assessed the behavioral tests including: 1) motor coordination has traditionally been assessed rotarod test [9], 2) the amphetamine-induced rotation test [10], and 3) spatial memory test with the Morris water maze device using the video recording [11], and measurement of overall home-cage activity levels to assess the protective effect of sake yeast on AS-induced learning and spatial memory impairment.

Oxidative stress

One day after the last treatment, rats were euthanized (by anesthesia with 0.64 mg/kg xylazine and 20 mg/kg ketamine (Alfasan, Woerden, the Netherlands)), and weighed on a 1-mg digital assay balance. The animal's head was then fixed; the surface of the skull was shaved and disinfected with betadine for 1 min described by Sokouti et al. [12]. The rat's brain was quickly transferred to a freezer at -80 °C for freezing. The samples were then homogenized in a saline phosphate buffer solution in a ratio of 1 to 10 and centrifuged at 12000× for 15 min (4 °C). 0.2 ml supernatant solution was selected for the determination of levels of malondialdehyde (MDA) and GPX by the instruction of the commercial kit (Colorimetric analysis). Blood serum samples were used to measure total antioxidant capacity (TAC) [13], making it possible to compare the results.

Histopathological studies

The brains were fixed in Bouin's solution for 24 h, dehydrated in a series of graded ethanol, and embedded in paraffin, Then they were cut into 5-µm sections using a Leica slicer (Leica, Inc., Germany) and stained with a hematoxylin and eosin kit (H&E) according to the manufacturer's instructions. An optical microscope was used to observe histopathological changes (Olympus, Tokyo, Japan). Ten visual fields per slide and six sections per group were selected randomly for analysis under 40× magnifications.

Western blot

The procedure for western blot analysis of proteins is as follows. Refer to previous reports [12]. Briefly, 1) brain tissue samples were ground and pulverized, and 2) were lysed in a buffer containing protease inhibitor and RIPA (Sangong Bio, Shanghai, China) (Table 1). A kit for protein concentration determination using BCA (Biyuntian, Shanghai, China) was used after completion at λ=630 nm. Goat anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. The remaining primary antibodies (Abs, Bioss) were purchased from Biosciences Pharmingen (San Diego, CA, USA). Then, the Electro-Chemi-Luminescence reagent was evenly coated on the NC membrane for 2 min. Images were collected and the target band was analyzed by optical density by Fluor Chem Q System (Alpha Innotech, CA, USA).

Statistical analysis

The biological replicates of each group of samples were greater than or equal to 6, and the data were expressed as " mean±S.D.". Statistical analysis was performed using the univariate analysis method of SPSS software (IBM Co., NY The results were expressed as mean ± standard error of the mean (SEM) and processed using a one-way analysis of variance (ANOVA) followed by Dunnett's new multiple range test, and values with $p < 0.05$ were considered as statistically significant.

RESULTS

Effects of administrated sake yeast supplement in drinking water on α-synuclein protein levels and oxidative stress indices in AS-induced rats are presented in Table 2 and Figure 1, receptively. Considering the vehicle group, AS-treated animals showed significantly ($P<0.05$) higher α-synuclein protein and MDA concentrations in brain tissue, while receptively impairing TAC and GPX levels in blood serum and brain tissue. Therefore, it can be concluded that AS has a strong damaging effect on the nervous system in male rats model. Meanwhile, sake supplementation can rescue the brain damage caused by this toxic metal, resulting in a reduction of α-synuclein protein and MDA levels, plus a considerable improvement in blood serum TAC consideration ($P<0.05$).

Table 1. The compounds of lysis bufferfor western blotting

Tris-HCL	EDTA	SDS	Sodium Deoxycholate	NaCl	Triton (NP40 (1%))	Protease inhibitor cocktail
500 µL, =pH7.8	0.004 g	0.01 g	0.024 g	0.081 g	10 µL	1 Tablet

Table ۲/ Effects of administrated of arsenic single or combined with sake in drinking water for ۳۰ consecutive days on α-synuclein protein levels and oxidative stress indices (mean ± SD)

Groups	α-synucleine/β-Actin	Total antioxidant status	Glutathione peroxidase	Malondialdehyde
Normal	0.35±0.03^{b}	0.63±0.05^{ab}	3.25±0.09^{b}	0.65±0.04^{c}
Arsenic (3 mg/kg)	0.61±0.31^{a}	0.32±0.01^{c}	3.01±0.01^{b}	1.57±0.01^{c}
Sake (45 mg/kg)	0.19±0.04^{b}	0.79±0.02^{a}	5.25±0.03^{a}	0.39±0.01^{a}
Arsenic + Sake	0.24±0.05^{b}	0.41±0.01^{c}	4.41±0.02^{b}	0.58±0.02^{b}

a-c Superscript s show significant differences in each row (P<0.05)

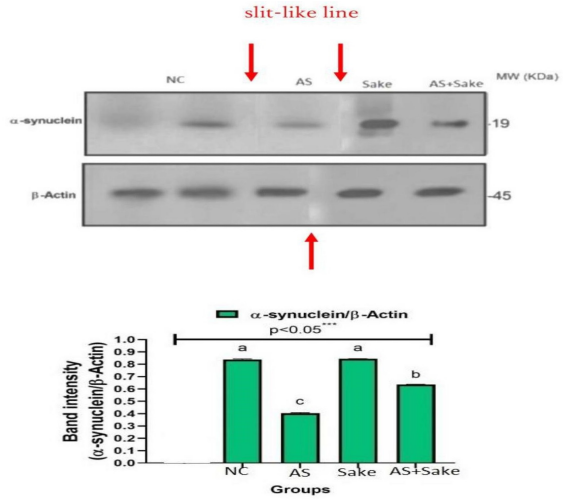

Figure 1. Changes in the concentrations of α-synuclein protein in arsenic induced rat; βactin was used as an internal control. (a-c)mean values with common letter(s) above bars do not differ significantly (p < 0.05)

The behavioral assay result indicated that sake supplementation could improve behavioral tasks (number of induced rotations, and duration of rod test), and spatial memory by increasing the number of rotations and rod test time in AS-treated groups (Table 3).

Figure 2 shows representative sections of brain tissue of rats in all groups. In control groups, the histopathological sections were normal (completely). Meanwhile, the administration of AS in drinking water caused failure to the number of dopaminergic neurons in the substantia nigra. In the sake and combination groups, treatments enabled the repair of the brain tissue by increasing the number of dopaminergic neurons in the substantia nigra.

DISCUSSION

Some results have proved that AS can cause severe oxidative damage to the brain cells and a major signature of brain tissue injury [3, 4]. Using chemical agents in the therapy of brain disorders may have some limitations due to frequent negative side effects. In our study, the decreased levels of various oxidative stress markers (including total antioxidant capacity and glutathione peroxidase activity) have been demonstrated in AS treatments that leading to the massive accumulation of MDA in brain tissue (a marker of lipid peroxidation). AS also diminishes the protective antioxidant enzyme contents thereby inflicting severe neuronal damage and directly affecting brain cells [14, 15]. All in all, the concentration of MDA in blood or tissue can be considered as an oxidative stress biomarker to assess the prognosis of brain injury stages. Due to the AS stress, the

Table 3. Effects of administrated of arsenic single or combined with sake in drinking water for 30 consecutive days on behavioral tasks and in spatial memory arsenic induced-male rats model

Days \ Groups	Test (turns/min)	Normal	Arsenic (3 mg/kg)	Sake (45 mg/kg)	Arsenic+Sake
1	Amphetamine rotation	0.00±00	0.00±00	0.00±00	0.00±00
	Rotarod	0.00±00	0.00±00	0.00±00	0.00±00
7	Amphetamine rotation	0.00±00	0.34±0.41	0.51±0.63	0.43±0.21
	Rotarod	0.00±00	0.00±00	0.00±00	0.00±00
14	Amphetamine rotation	0.00±00^{a}	3.53±1.16^{b}	3.63±1.16^{b}	3.59±1.06^{b}
	Rotarod	0.00±00^{a}	3±00^{a}	3±00^{a}	3±00^{a}
21	Amphetamine rotation	0.00±00^{a}	2.66±1.36^{b}	3.26±0.96^{b}	2.81±0.21^{b}
	Rotarod	0.00±00^{a}	2.41±0.58^{ab}	2.18±0.59^{b}	2.33±0.11^{b}
28	Amphetamine rotation	0.00±00^{a}	0.53±0.44^{ab}	0.31±0.21^{ab}	0.28±0.11^{ab}
	Rotarod	0.00±00^{a}	1.55±0.62^{b}	0.30±0.42^{a}	0.29±0.09^{ab}
Spatial memory	Time to the quarter (sec)	10.8±0.15^{e}	10.61±0.19^{c}	12.487±0.14^{e}	11.04±0.25^{d}
	Distance travel (cm)	132662±159^{e}	104837±2579^{c}	133431±141^{e}	43121±2214^{d}

a-d Superscript s show significant differences in each row (P<0.05)

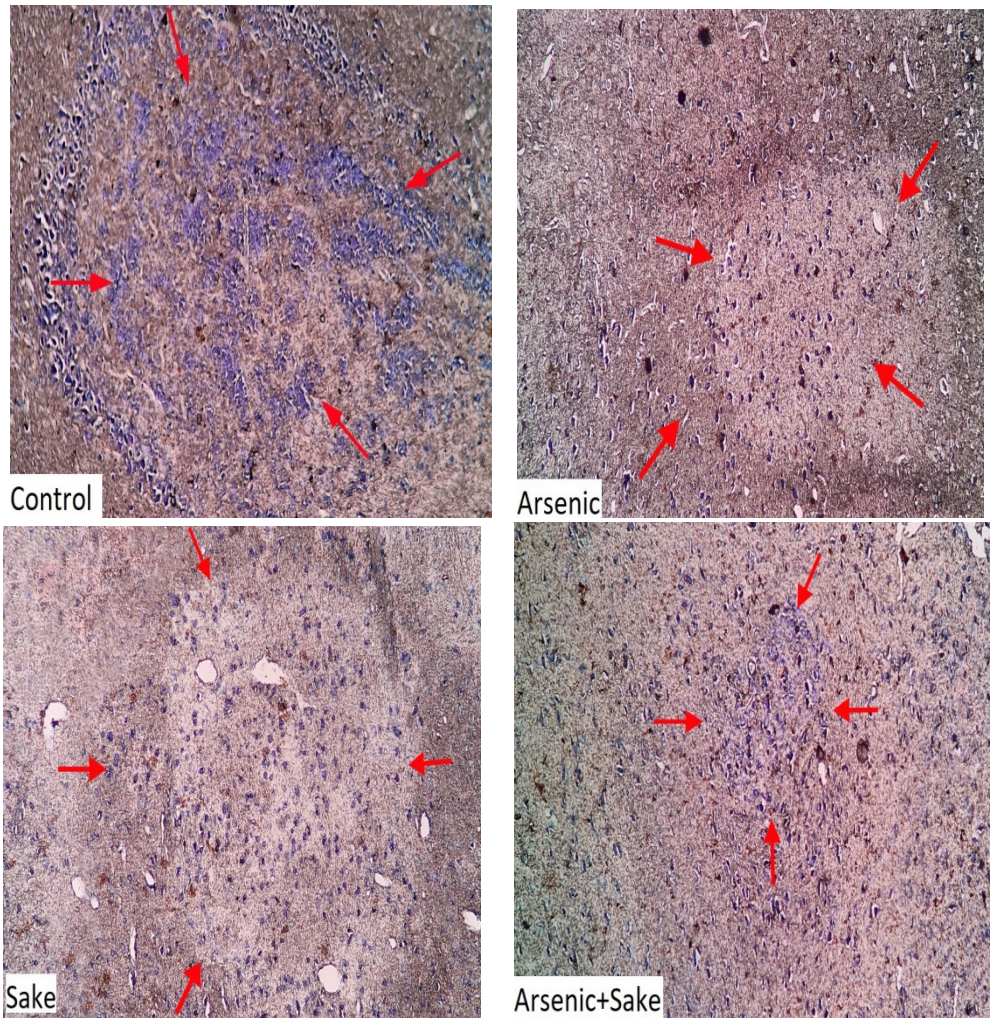

Figure 2. Brain histopathological structure and the regeneration of dopaminergic neurons in the substantia nigra of rats after 30 days simultaneously mentioned below treatments (through oral gavage)

reactive-oxygen species (ROS) are highly produced in the brain tissue [16], which leads to severe oxidative damage. Excessive accumulation of ROS can result in lipid and protein peroxidation, and fragmentation of DNA, which later develops into cell death [17]. In some in-vitro cell line models, AS was found to activate pro-inflammatory factor, NF-κB signaling, mediated through oxidative stress [18, 19].

In more detail, we show that AS is responsible for the death of dopaminergic neurons by diminishing the number of substantia nigra neurons, but its mechanisms of action must be assessed in future studies. The brain is particularly vulnerable to AS-induced deleterious effects because AS readily evades the blood-brain barrier [20]. Recently, Kanungo et al. [21] for the first time showed that inorganic AS alters the development of motor neurons and in dopaminergic zebrafish larvae and the latter occurs through the Sonic hedgehog (Shh) pathway.

Some natural agent's likely promising alternatives in the treatment of brain disorders. Monoi et al. [22] have reported that sake yeast intake will have beneficial effects on maintaining metabolic, vascular and brain functions. The proteinogenic amino acid profile in sake yeast like phenylalanine is used to produce important signaling molecules such as dopamine and epinephrine via tyrosine biosynthesis [23, 24]. In confirming to the above reports, the current report sake treatments by increasing the number of dopaminergic neurons in the substantia nigra enabled the repair of the brain tissue due to their pharmaceutical properties and lower toxicity by: 1) may have further improved oxidative stress defence mechanism, 2) may have further reduced the scavenging of AS-generated ROS.

The current results showed that the daily sake yeast intake had ample potential for improving oxidative changes. They suggest the abrogation of oxidative changes and modulation of behavioral tasks as a mechanism of action sake yeast. These results are in line with Davoodi et al. [5]. Accordingly, it was inferred that strategies aimed by sake yeast for 30 consecutive days at reducing oxidative stress and lipid peroxidation could be potential therapeutic targets for the treatment of AS neurotoxicity in the male rats model. For future studies, pharmacokinetic analysis is significant to understand how long sake yeast affects brain function after

intake.

Our results indicate that exposure to AS impairs α-synuclein protein concentration in the brain tissue affecting spatial memory. This impairment is associated with increased oxidative damageto nervous system. Spatial memory increases the likelihood of falls and deficits reduce skilled walking performance in older adults [25]. Sake yeast ameliorates AS-induced neurotoxicity through reduction of α-synuclein protein levels, plus a considerable improvement in blood serum TAC consideration in rat brains.

Our results indicated that AS develops neurotoxicity and causes brain dopaminergic neurons in the substantia nigra alterations in rat models, which is a potential mechanism of AS-induced neurotoxicity. Therefore, assume that the daily sake yeast intake had ample potential for improving oxidative changes.

CONCLUSION

Sake yeast proved a protective effect on neurodegenerative toxic morphological changes induced by AS in dopaminergic neurons of substantia nigra in rat models.

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