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Silymarin attenuates aspartame-induced variation in mouse behaviour, cerebrocortical morphology and oxidative stress markers

Adejoke Yetunde Onaolapo[a], Saratu Zayid Abdusalam[b], Olakunle James Onaolapo[b,*]

[a] Department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria
[b] Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria

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ABSTRACT

Objective: We assessed the impacts of silymarin co-administration on aspartame-induced changes in novelty-induced behaviours, memory, anxiety-related behaviours, cerebral antioxidant status and histomorphology in mice.
Method: Six groups of mice were administered vehicle (distilled water), silymarin (25 mg/kg), aspartame (at 160 or 320 mg/kg), and silymarin (25 mg/kg) co-administered with aspartame at 160 or 320 mg/kg daily for 21 days, via an oral cannula. Behaviours were assessed after the first and last dose of treatment. Animals were sacrificed thereafter. Brain homogenates were used to assess antioxidant status; while sections of the cerebral cortex were processed for routine histology.
Result: Repeated co-administration of silymarin with aspartame resulted in significant suppression of horizontal locomotion and rearing, while grooming behaviour was enhanced; when compared to aspartame alone. Spatial working-memory showed significant improvement only after acute co-administration, while anxiety-related behaviours were reduced after repeated administration of both silymarin and aspartame. Cerebral cortical morphological integrity was better preserved, and astrocytic reactivity reduced with silymarin co-administration. Brain activity of superoxide dismutase and nitric oxide levels were decreased, while glutathione peroxidase activity was increased, when compared to levels seen with aspartame alone.
Conclusion: The study shows that co-administration of silymarin with aspartame was associated with significant attenuation of central effects, when compared to administration of aspartame alone.

1. Introduction

Silymarin is a polyphenolic antioxidant complex that is derived from the seeds and fruits of *Silybum marianum*, also known as 'milk-thistle' plant. Milk-thistle is an ancient medicinal plant that had been in use for centuries; its use range from management of liver and gallbladder disorders, to hepatoprotection in the advent of a snake bite or insect sting, and protection from mushroom or alcohol poisoning [1]. Silymarin, the crude commercial product of milk thistle [2] is a complex of seven flavonolignans (the most important being silybin, isosilybin, silydianin, and silychristin) [3–6] and one flavonoid (taxifolin) [5] that comprises 50% to 80% of the extract [2–6]. Silymarin now has worldwide use as a complementary medicine alternative [5].

Silymarin is known to have antioxidative, antifibrotic, anti-inflammatory and immunomodulating properties [4]. It acts as an antioxidant by scavenging free-radicals and increasing the concentration of glutathione [7,8]. Results from a number of studies have shown that silymarin is non-toxic at pharmacological doses [4,8–11]. In contemporary medicine, silymarin is used in the management of alcoholic liver disease, liver cirrhosis, hepatitis and radiation injuries [12]; and presently, milk-thistle extract is marketed (under different trade names) as silymarin and/or silybinin tablets or capsules (which is reported to have improved bioavailability and standardization of content).

Studies on silymarin have used either the milk-thistle extract [13,14], or standardized formulations [15,16]. Regardless of the form of silymarin used, reports of its hepatoprotective [17], nephroprotective [18], antioxidant [19], anticancer [20], cardioprotective [16,18], or neuroprotective effects [13] remain undisputed. The

* Corresponding author.
E-mail addresses: olakunleonaolapo@yahoo.co.uk, adegbayibiy@yahoo.com (O.J. Onaolapo).

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effects seen with silymarin administration have been attributed to different mechanisms; one of the most important, is that silymarin scavenges free-radicals at various steps in the arachidonic-acid cascade, via the cyclooxygenase and lipoxygenase pathways [21]. It also modulates the immune system via its inhibitory effects on neutrophil migration and the mobilisation of mast cells [22]. Silymarin is known to interfere with the expression of the cell-cycle regulators and proteins involved in apoptosis [5].

A few studies have reported silymarin's ability to cross the blood-brain barrier, and thereby modulate brain oxidant/antioxidant status [23–25] or affect behaviour [26] and/or morphology [27], via its interaction with brain neurotransmitters and/or their receptors. Considering the documented neuroprotective potentials of silymarin, we set out to assess the effects of silymarin at a fixed dose of 25 mg/kg [28,29] on behavioural and morphological changes we had earlier reported after administration of aspartame at 160 mg/kg [30]. The objectives of this study were two-fold: first, to determine the effects of repeated administration of silymarin (at a fixed dose of 25 mg/kg) alone, or co-administered with aspartame at 160 or 320 mg/kg on open-field novelty-induced behaviours, spatial working-memory, anxiety-related behaviours, brain antioxidant status (superoxide dismutase/glutathione peroxidase activities and nitric oxide level), and cerebral cortical morphology/morphometry in mice; then, to compare the results with those obtained when aspartame is administered alone.

Aspartame is an odourless, intensely-sweet, white, crystalline powder which is an important component of a number of low-calorie and sugar-free food [31]. In a recent study from our laboratory [30], we demonstrated that repeated administration of aspartame to mice at 160 mg/kg/day, (approximately 13 mg/kg human equivalent dose; which is within the recommended daily allowance of 40 mg/kg in Europe and 50 mg/kg in America) induced changes in behaviour and cerebral cortex morphology [30]. The projected maximum consumption of aspartame in most human populations has been reported to range from 22 to 34 mg/kg body weight/day [32]. However, more recent observations have revealed that aspartame is slowly making its way into every-day products; thus, ingestion of aspartame is extending to individuals who are possibly unaware of their consumption of aspartame-containing products [33], and as such cannot regulate their consumption. This means that the recommended maximum daily intake may easily be reached and exceeded; and thus, people may be at risk of possible behavioural or morphological changes that have been associated with consumption of high doses of aspartame, as seen in rodents. Hence, the rationale for this study was to examine (in mice) the effects of silymarin on the changes that we reported were associated with repeated administration of aspartame at increasing doses. We tested the hypothesis that, acute or repeated oral administration of silymarin could significantly attenuate changes in open-field novelty-induced behaviours, memory, anxiety-related behaviours, oxidative stress, morphology and morphometry of the cerebral cortex known to be associated with consumption of aspartame.

2. Materials and methods

2.1. Animals

Male Swiss mice (Empire Breeders, Osogbo, Osun State, Nigeria) weighing 20–22 g each were used for this study. Mice were housed in groups of five in enriched (transparent plastic cages with exercise ladders, shredded-paper beddings, artificial burrows made of plastic tubes and platforms) cages located in a temperature-controlled quarters (22–25 ° Celsius) with 12 h of light daily. All animals were fed commercial (Top Feeds®, Premier feeds Ltd, Nigeria) standard chow (Calories: 29% protein, 13% fat, 58% carbohydrate) from weaning. Animals were administered food and water *ad-libitum* except during the behavioural tests. All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

Table 1
Experimental groups and number of animals in each test group (n).

Groups		Open field	Y- maze	EPM
VEH(10 ml/kg)	n = 10	yes	yes	yes
SILY (25 mg/kg)	n = 10	″	″	″
ASP (160 mg/kg)	n = 10	″	″	″
ASP (320 mg/kg)	n = 10	″	″	″
SILY + ASP (160 mg/kg)	n = 10	″	″	″
SILY + ASP (320 mg/kg)	n = 10	″	″	″
TOTAL	60	–	–	–

VEH: Vehicle, SILY: Silymarin, Asp: Aspartame, EPM: Elevated plus maze, n = number of animals/group.

2.2. Drugs

Aspartame tabletop sweetener (99.9% purity, NutraSweet® Nutra Sweet Company, Illinois, USA) and Silymarin (Silybon-70®, Micronova Pharmaceutical Industries Ltd, Lagos, Nigeria) were sourced from the pharmacy. Doses of silymarin or aspartame were calculated by dissolving measured quantities in distilled water, and they were administered at a volume of 10 ml/kg.

2.3. Experimental method

Sixty male Swiss mice were randomly assigned into 6 groups of ten mice each. Animals in respective groups (Table 1) received vehicle (distilled water) at 10 ml/kg, silymarin at 25 mg/kg, aspartame at 160 mg/kg and 320 mg/kg or silymarin co-administered with aspartame at 160 mg/kg or 320 mg/kg daily for 21 days, via an oral cannula. Doses of silymarin [28,29] and aspartame [30] were based on previous studies, and were calculated after dissolution in distilled water. Behavioural tests were conducted on days 1 (acute) and 21 (repeated). Immediately after the last behavioural test (day 21), animals were euthanized; their brains were removed, sectioned, and either homogenised for estimation of antioxidant status or processed for general histology.

2.4. Behavioural tests

Behavioural tests were conducted on days 1 and 21; thirty minutes after administration of treatment. Tests were conducted in a quiet room between the hours of 8 a.m. and 2 p.m. On each of the test days, mice were transported in their home-cages to the behavioural testing laboratory, allowed 30 min to acclimatize, and then administered drug or vehicle. Animals were allowed to explore the open-field for 20 min, and the Y-maze and elevated plus-maze for 5 min each. At the beginning of the behavioural tests, each animal was placed in the apparatus and its behaviour videotaped for subsequent analysis. After testing, each mouse was removed from the maze and returned to its home cage; all interior surfaces were cleaned thoroughly with 70% ethanol, and then wiped dry to remove any trace of odour. The behavioural parameters were later scored by two independent observers who were blind to the groupings.