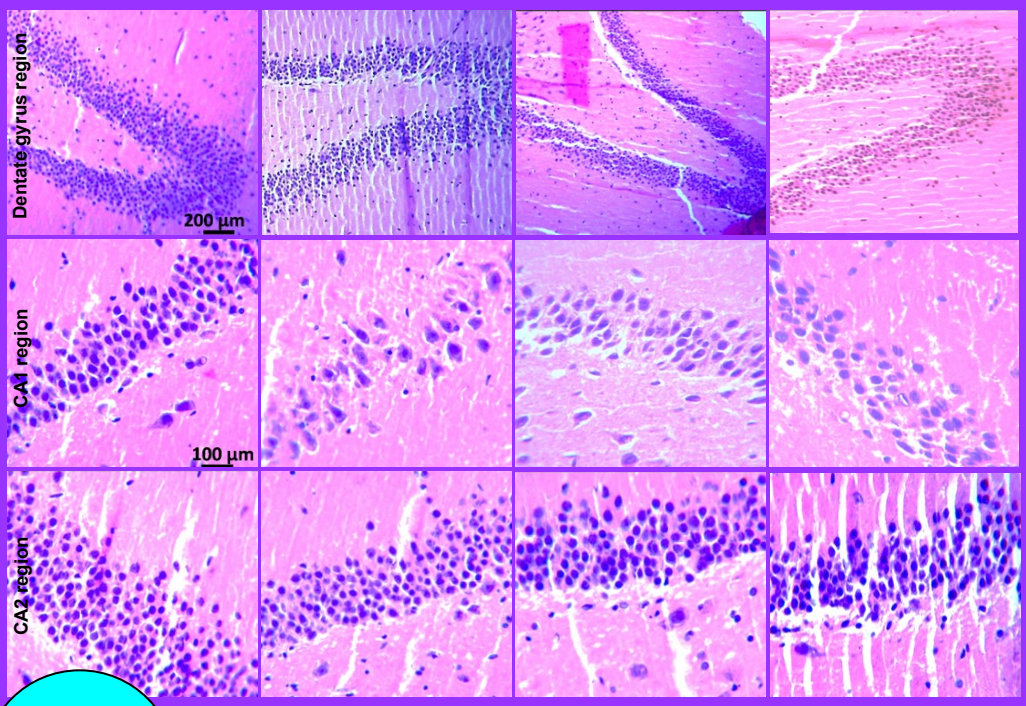

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Protective effects of resveratrol in high fat diet- and metals (Al, Pb, As)-induced metabolic and cognitive impairments

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Abstract

This study evaluated the neuroprotective role of resveratrol in rats co-exposed to high fat diet and metals (aluminum, lead, arsenic) for 60 days. Rats (male, 10–12 weeks age, 180–250 g) were divided into control, modified meal (high fat diet + metals in drinking water), and resveratrol-treated groups (5 and 20 mg/kg). Behavioral tests (Morris water maze, Y maze, social interaction) revealed impairments in memory and reduced sociability in the modified meal group. Biochemical and histological analyses showed dysregulated lipid profiles, elevated blood glucose, signs of liver and kidney dysfunction, neuronal loss, and structural damage in the hippocampus. Resveratrol treatment, particularly at 20 mg/kg, significantly restored cognitive performances by increasing time spent in the target quadrant (78.9 ± 2.0; $p<0.001$), improving spontaneous alternation performance (66.6 ± 2.3; $p<0.001$), and improved metabolic dysfunction. These findings suggest resveratrol's therapeutic promise in mitigating the neurotoxic effects of high fat diet and metals.

Introduction

The rapid rise of industrialization and globalization has significantly transformed dietary habits worldwide, contributing to a growing dependence on highly processed foods rich in saturated fats. This shift of consuming a high fat diet is particularly evident in developing countries, resulting in increased rates of hypertension, type 2 diabetes, obesity, and neurodegenerative conditions (Wali et al., 2020; Clemente-Suarez et al., 2023). Alarmingly, more than two-thirds of adults in the USA and over half in Europe are linked to excessive high fat diet consumption (Alwan, 2011).

Substantial evidence suggests that high fat diet not only disrupts metabolic balance but also impairs brain function, particularly hippocampus-dependent learning and memory (Pistell et al., 2010; Francis and Stevenson, 2013). High fat diet is also reported to promote neuroinflammation, oxidative stress and decrease levels of brain-derived neurotrophic factor, all these factors as a result accelerate neurodegeneration (Morrison et al., 2010; Tan and Norhaizan, 2019). Furthermore, high fat diet interferes with the cholinergic system by decreasing acetylcholine levels causing cognitive decline (Iqbal and Ahmed, 2019).

Exposure to environmental toxins, especially metals such as aluminum, lead, and arsenic is an emerging issue (Mitra et al., 2022). These metals enter the body through drinking water and food. The metabolism and excretion of metals from the body become extremely challenging, leading to increased buildup in many vital organs including the brain, which result impacts cognition (Raehsler et al., 2018, Briffa et al., 2020). Chronic and prolonged exposure to metals causes obesity (Park et al., 2017, Wang et al., 2018), cardiovascular and respiratory diseases (Lamas et al., 2016, Wu et al., 2019),

and autism spectrum disorder (Bjorklund et al., 2018, Iqbal et al., 2018). Particularly, arsenic and lead, commonly found in the food chain, have been implicated in contributing to metabolic dysfunction through their toxic effects on cellular and systemic processes (Liu et al., 2020).

Although individual metal toxicities are well documented, real-world exposure typically involves complex mixtures of metals. Such combined exposure causes severe health issues (Cathe et al., 2017, Freire et al., 2018), such as disruption of the blood-brain barrier, enhanced generation of reactive oxygen species (ROS), (Shukla et al., 1996, Lopez et al., 2006) and cognitive impairments (Mazzocco et al., 2020). Different epidemiological studies from the US, Korea, China, and Taiwan confirm that humans are frequently exposed to different metal mixtures, which have been associated with a vast range of metabolic disorders, cardiovascular diseases, and neurodegeneration (Grundy et al., 2005, Rotter et al., 2015, Wang et al., 2018).

Given this growing health burden, there is an urgent need to identify therapeutic agents capable of counteracting the effects of high fat diet and metals. Resveratrol, a natural polyphenol found in berries, red grapes, and peanuts has shown promising neuroprotective and metabolic benefits (Baur and Sinclair, 2006, Meng et al., 2021). Its antioxidant and anti-inflammatory properties make it an ideal therapeutic candidate for neurodegeneration and oxidative stress (Alarcon De La Lastra and Villegas, 2005; Bastianetto et al., 2015). Furthermore, it showed protective effects against aggregated beta-amyloid neurotoxicity (Morris, 2009).

Based on these observations, this study aimed to investigate the protective effects of resveratrol.

Materials and Methods

Chemicals and reagents

Lead acetate, sodium arsenate, paraformaldehyde, Mayer's hematoxylin, and eosin were purchased from Sigma-Aldrich, USA. Aluminum chloride was purchased from Scharlau Chemical Company, Spain. Resveratrol was bought from TCI, America, Product Code: R0071.

Animals and exposures

Rats (male, Sprague Dawley, 10–12 weeks age, 180–250 g) were obtained from the Atta-ur-Rahman School of Applied Biosciences animal house facility and were housed in standard plastic rodent cages groupwise and were kept under regulated temperature 25 ± 2°C, the environment was well maintained with natural light-dark cycle (Iqbal et al., 2016).

Standard feed and water were provided *ad libitum*. Metals were added to the water for animal exposure and the high fat diet group had 40% of crude fat added to the feed as mentioned earlier (Iqbal and Ahmed, 2019). High fat diet was prepared by mixing normal feed and tallow at the ratio of 60:40. Fat was heated and then filtered to remove undissolved parts and mixed with feed. For treatment purposes, resveratrol powder was mixed in the feed. Diet was provided in the form of pellets for 60 treatment days. Female rats were excluded from this study because fluctuations in hormone levels could lead to variability in data interpretation and increase overall result variability.

High fat diet and metals exposure and resveratrol treatment study design

The study comprised 60 days trial. Rats were divided into four groups. Each group composed of 10 male rats. Group I comprises of the control rats. These rats were fed with normal feed and water for 60 days. Group II exposed to a modified meal (high fat diet + metals- 25 mg/kg $AlCl_3$, 25 g/kg $PbCl_2$ and 25 mg/kg arsenic in drinking water). The high fat diet group had 40% of crude fat added to the feed. Group III received a modified meal and resveratrol 5 mg/kg (mixed in feed). Group IV received a modified meal and resveratrol 20 mg/kg. At the end of treatment, rats underwent behavioral analysis and were later dissected for biochemical and histological analyses. Animals were subjected to deep anesthesia after a 12-hour fasting period for lipid profiles. Plasma lipid profile, liver function, renal function, and HbA1c levels were measured from freshly collected blood. Collected blood samples (2 mL) were centrifuged at 2,000 × g for 15 min at 4°C. For brain tissue preparation and preservation, rats were anesthetized with intraperitoneal ketamine injection (60 mg/kg) and then transcardial perfusion was performed first with PBS and then followed by 4% paraformaldehyde.

Behavioral analysis

Animals were shifted to the behavior room 30 min before the start of behavior. The time frame for all the behavior testing was between 10 AM to 5 PM. Video recording of all the behavior tests was carried out manually which were later analyzed, and data was plotted manually using GraphPad prism software.

Morris water maze task for learning and memory

This test was performed to analyze long-term memory. The testing apparatus comprised a pool with a hidden platform. The whole procedure involved five trials per day over five days, following the previously described protocol with slight modifications (Zahoor et al., 2024). In each trial, rats were released from different starting points while the platform location remained constant. This routine was repeated for five consecutive days and on the sixth day, a probe trial was conducted by removing the platform. During this trial, the number of platform area crossings, number of entries into the target quadrant, and the time spent in the target qua-

Box 1: Y maze test

Principle

The Y maze test (spontaneous alternation test) was performed to assess the hippocampus-based learning and memory, and analyzed the short-term or working memory.

Apparatus

The apparatus consisted of a Y-shaped maze having three arms of the same size.

Procedure

Step 1: Before the start of the Y maze task, arms of the maze were labelled as start, other and novel arm.

The test was conducted in two sessions with 30 min inter-trial interval in between.

Step 2: In the first session, the labelled novel arm was blocked with a wooden door and the rat was placed and allowed to explore the "start" and "other" arms for 15 min.

Step 3: After a break of 30 min, before starting the second session, the wooden door was removed to give access to the novel arm and the rat was allowed to freely explore the Y maze for 5 min.

Step 4: The trial test was recorded using a video camera and the videos were analyzed offline.

Step 5: The time spent in each arm and number of entries in each arm were recorded.

The task was performed following the protocol with slight modifications. Spontaneous alternations were defined as when a rat explored all three arms in a sequential manner, such that it explored arms 1, 2 and 3. Alternative arms repeats were defined as when the rat did not follow the sequence as mentioned in spontaneous alternations. Percent alternation assesses spatial working memory by measuring the animal's ability to alternate between all three arms.

The Y-maze apparatus was thoroughly cleaned with 70% ethanol and allowed to dry before each trial. Videos were recorded manually, and data were analyzed manually. The experimenter remained present for safety, timing, and observation, while minimizing interference with the animals' behavior.

Reference

Zahoor et al., 2024

drant were recorded.

Social preference and novelty test

This test studied social memory and affiliation as described previously with slight modifications (Ishaq et al., 2023). It was carried out in a three-chambered glass box. The animal could navigate easily through the three openings. There were two sessions in this test: Session 1 where the animal's social preference was assessed, and Session 2 where the animal's novelty was assessed. Each session was for 10 min and between both sessions, there was 20 min. Both the sessions were recorded using a camera.

Biochemical testing/serum profiling

Animal blood samples were collected in heparin tubes. Samples were then centrifuged at 3,000 x g for 10 min. Liver function tests were measured by checking the levels of alanine aminotransferase, alkaline phosphatase, and total bilirubin. Cholesterol levels were measured using a UniCel DxC800 Synchron chemistry system (Bekman, USA). Kidney profiling was done to check kidney dysfunction. To assess diabetic levels HbA1c testing was performed. The results of these tests can give an approximate estimation of the metabolic health of the animals.

Histological examination

Tissue preparation: The rat brain tissue was then extracted and kept in 4% paraformaldehyde solution for another 24-48 hours at 4 °C. Later the brain tissue was fixed and paraffin-embedded, and the tissue was sliced into 3 µm coronal sections.

Hematoxylin and Eosin (H&E) staining: H&E staining of paraffin-embedded brain sections was performed to assess neurodegeneration. The rat brain sections were first deparaffinized in xylene for 5 min, then rehydrated in isopropanol for another 5 min, and stained with Mayer's hematoxylin for 8 min. After this, a warm water rinse was given and destained in 95%ethanol for 2 min followed by eosin counterstaining for 30 sec as mentioned earlier with slight changes to the protocol (Shaibah et al., 2016). Microscopic examination of prepared slides was carried out under a microscope (Optika B-150, Italy), and images were captured at 40x magnification using Optika Vision Lite 2.1 software.

Quantitative analysis of neuronal cell density: Quantitative cell analysis was conducted in the hippocampal regions (dentate gyrus, CA3, CA2, and CA1 regions). Cell counting was performed within an area of 2450 μm^2 by randomly selecting three sites per region. The average cell count values for each hippocampal region were calculated and presented graphically.

Statistical analysis

Statistical analysis was performed using GraphPad Prism v7.0. Normality was assessed via the Shapiro-Wilk test (n=10/group) and confirmed with histograms and Q-Q plots. As data were normally distributed, parametric tests were applied. Behavioral data (MWM, social interaction, Y-maze) were analyzed using two-way repeated-measures ANOVA with Bonferroni post hoc tests. Histological and biochemical data were evaluated by one-way ANOVA and presented as mean ± SEM. Significance was set at $p<0.05$.

Results

Two-way repeated measures ANOVA revealed that all groups displayed marked improvement in locating the hidden platform over successive training days. However, the modified meal group showed impaired learning, with a significantly higher mean latency (20.1 ± 0.9 sec) compared to the controls (10.7 ± 0.9 sec) on the final day (Figure 1A). In contrast, treatment with resveratrol (5 and 20 mg) improved performance, as indicated by reduced latencies (12.2 ± 0.6 sec and 12.9 ± 0.8 sec), suggesting enhanced spatial memory acquisition.

The control group also spent significantly more time in the target quadrant (78.1 ± 2.0 sec), indicating better memory retention than the modified meal group (50.2 ± 1.7 sec). Resveratrol-treated groups (5 and 20 mg) showed noticeable improvement, spending more time in the target quadrant (82.6 ± 2.9 sec and 78.9 ± 2.0 sec, respectively; p<0.001, Figure 1B). Resveratrol also enhanced the target quadrant entries (Figure 1C) and platform crossings (Figure 1D), supporting its memory-enhancing effects.

Y maze test

The modified diet group showed fewer novel arm entries (2.5 ± 0.2) than controls (8.4 ± 0.3), while resveratrol treatment (5 and 20 mg) increased the number of entries (3.8 ± 0.2 and 5.4 ± 0.3 respectively), indicating improved spatial recognition (Figure 2A). Similarly, resveratrol-treated groups spent more time in the novel arm (54.4 ± 2.9 sec and 67.4 ± 1.8 sec) compared to the modified diet group (40.0 ± 1.6 sec; p<0.001), indicating enhanced spatial recognition (Figure 2B).

The spontaneous alternation performance (%) data showed cognitive impairment (33.2 ± 3.0 %), while resveratrol treatment (5 mg: 45.9 ± 2.4 %; 20 mg: 66.6 ± 2.3 %) improved the performance close to the levels of control (68.2 ± 2.8 %; Figure 2C). The modified meal group also showed increased AAR (50.8 ± 3.6 %) and SAR (6.8 ± 0.6 %), indicating memory impairment. Resveratrol treatment (5 mg and 20 mg) significantly reduced AAR (32.0 ± 1.0 %; 33.5 ± 1.3 %) and improved performance (p<0.001; Figure 2D-E).

Social preference and novelty test

In session 1, the modified meal group spent less time with the novel rat (234.5 ± 3.7 sec) than the control (381.9 ± 11.8 sec) and resveratrol-treated groups (5 mg: 312.5 ± 3.9 sec; 20 mg: 323.9 ± 4.8 sec), indicating reduced social preference for interaction. (Figure 3A, 3C). In session 2, the modified meal group spent significantly less time with the stranger rat (8.9 ± 2.0 sec) and more with the familiar rat (57.8 ± 2.7 sec), indicating impaired social behavior. Resveratrol treatment (5 and 20 mg) showed increased interaction with the stranger rat (60.8 ± 1.9 sec; 73.0 ± 2.3 sec) and reduced time with the already familiar rat, reflecting improved social memory (Figure 3, session 2).

Blood glucose test

HbA1c (%) levels were elevated in the modified meal group (4.1 ± 0.5%) compared to the controls (2.4 ± 0.3%, p<0.05). Resveratrol treatment at 5 mg (3.3 ± 0.3%) and 20 mg (3.0 ± 0.2%) reduced the levels to values comparable to the control group (Table I).

Lipid profile

Lipid profiling revealed significant dyslipidemia in the

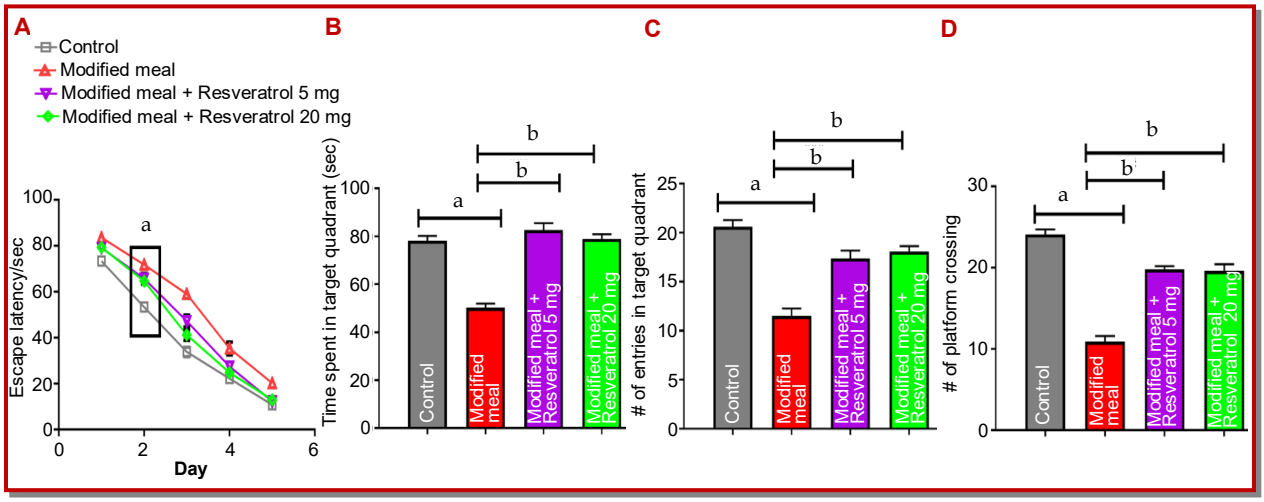

Figure 1: Effect of resveratrol on learning and memory using Morris water maze test. The graph demonstrates the escape latency (sec) to assess the reference memory formation and learning among the control, modified meal, and modified meal + resveratrol (5 mg or 20 mg) using two-way ANOVA followed by Bonferroni's multiple comparison test, training (A). Probe trial (B-D). The bar charts depict (B) the time spent in the target quadrant (sec), by all groups, (C) the number of entries in the target quadrant, (D) shows the number of platform crossings. The error bars are representatives of mean ± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with [a]p<0.001 significant values; [b]p comparison between modified meal and treatment group

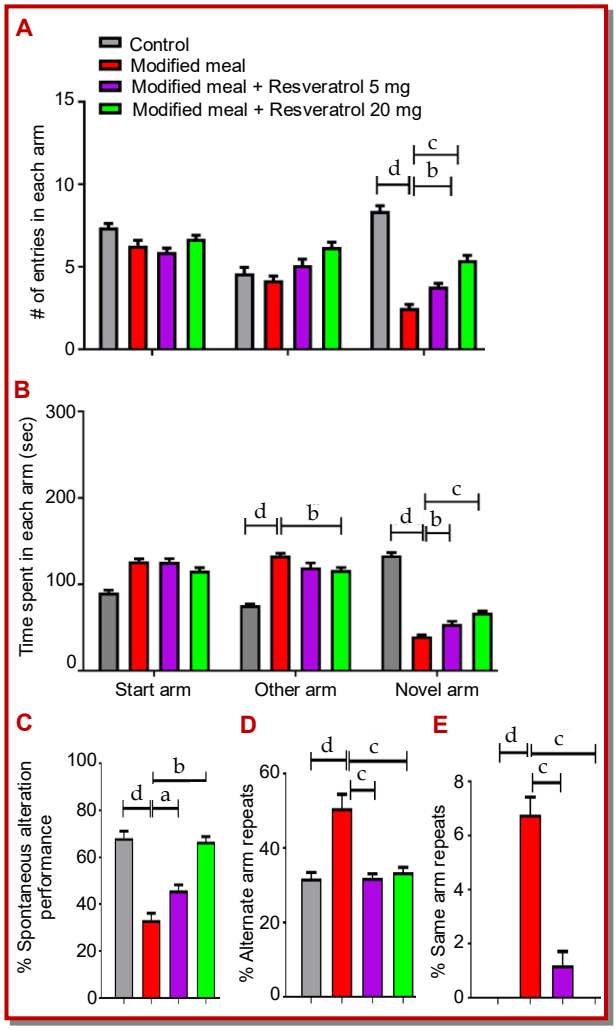

Figure 2: Effect of modified meal on animal performance, % spontaneous alternation in Y-maze test (A-C) and memory impairment (D-E). The bar charts depict the number of entries in each arm (A), time spent (sec) in each arm (B), %spontaneous alternation (C), %alternate arm repeats (D), and % same arm repeats (E) in control (first bar from left), modified meal (second bar), modified meal + resveratrol (5 or 20 mg, third and fourth bar). Error bars are represented as mean ± SEM, for one-way ANOVA, followed by Bonferroni's multiple comparison test. [a]p<0.05, [b]p<0.01, [c]p<0.001 and [d]p<0.001 are the significance values

modified meal group, with elevated total cholesterol (169.3 ± 27.8 mmol/L), triglycerides (253.5 ± 15.3 mmol/L), and LDL (61.8 ± 6.5 mg/dL), alongside reduced HDL (18.2 ± 0.9 mg/dL) compared to the controls. Resveratrol treatment (5 and 20 mg) markedly improved lipid profiles, lowering total cholesterol, triglycerides, and LDL, while restoring HDL levels to normal values close to the control group (Table I).

Liver function damage upon modified meal exposure

The modified meal group showed elevated markers of liver dysfunction, including alanine aminotransferase (112.0 ± 13.1 U/L), alkaline phosphatase (660.7 ± 62.9 U/L), and bilirubin (0.7 ± 0.1 mg/dL), compared to the controls. Resveratrol treatment reduced alanine aminotransferase and alkaline phosphatase levels in both 5 and 20 mg/kg groups, with greater effect at the higher dose. Bilirubin levels were partially normalized but not significantly different from the modified diet group (Table I).

Kidney profiling

Kidney function markers were significantly impaired in the modified meal group, with elevated blood urea (64.5 ± 5.8 mg/dL) and creatinine (1.4 ± 0.3 mg/dL) compared to controls (21.0 ± 1.1 mg/dL and 0.5 ± 0.1 mg/dL, respectively). Resveratrol treatment (5 and 20 mg/kg) significantly reduced blood urea and restored creatinine levels to near-control values, indicating renal protection (Table I).

Histological analysis in the hippocampal area

H&E and cresyl violet staining were used to assess the hippocampal neurodegeneration per unit area (μm²) across dentate gyrus, CA1, CA2, and CA3 regions (Figure 4). The modified meal group showed significant neuronal loss in the dentate gyrus (4.0 ± 0.5) compared to controls (8.3 ± 0.4; p<0.001). Resveratrol treatment improved cell counts to 6.5 ± 0.4 (5 mg; p<0.01) and 8.2 ± 0.3 (20 mg; p<0.001), indicating neuroprotection (Figure 4).

In the hippocampal CA1 region, the modified meal group showed a reduced cell count (3.0 ± 0.36) compared to controls (9.0 ± 0.36; p<0.001). Resveratrol treatment improved cell counts to 5.5 ± 0.4 (5 mg; p<0.01) and 7.3 ± 0.5 (20 mg; p<0.001), with the higher dose showing greater neuroprotection (Figure 4).

In both CA2 and CA3 regions, the modified meal group showed significant neuronal loss (CA2: 4.7 ± 0.4; CA3: 3.5 ± 0.4) compared to controls (CA2: 9.7 ± 0.3; CA3: 9.8 ± 0.7; p<0.001). Resveratrol treatment increased cell counts in CA2 and CA3 regions, with higher doses showing greater effect in CA2 (6.5 ± 0.4 at 5 mg; 8.8 ± 0.5 at 20 mg) and similar improvement in CA3 (7.2 ± 0.3 and 7.2 ± 0.5), indicating neuroprotection (Figure 4).

Discussion

This study evaluated the protective effects of resveratrol against cognitive, metabolic, and histopathological impairments induced by combined exposure to a high fat diet and metal mixture in male rats. Co-exposed animals showed significant deficits in spatial learning, working memory, and social behavior, along with metabolic dysfunction, including impaired glucose regulation, dyslipidemia, hepatic and renal damage, and hippocampal neurodegeneration. Resveratrol, particularly at 20 mg/kg, significantly improved cognitive performance, normalized metabolic and organ

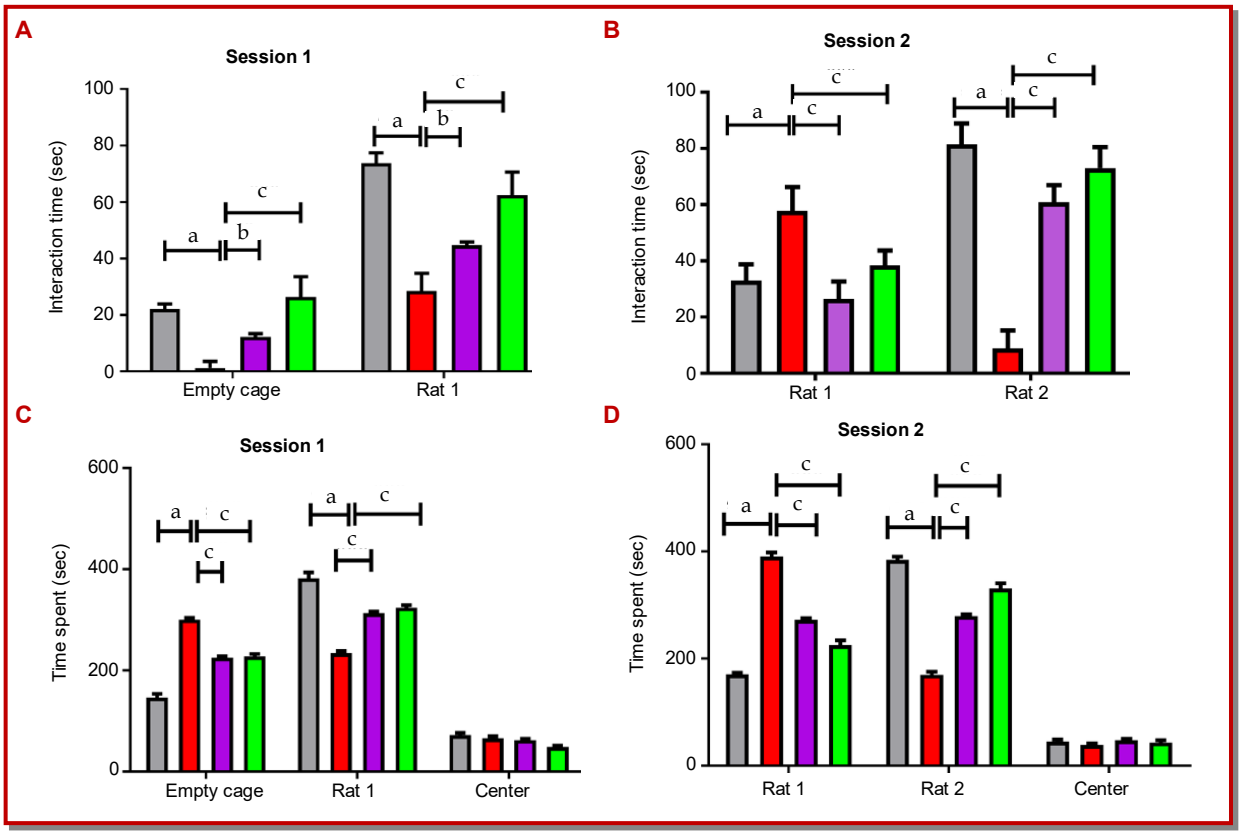

Figure 3: Effect of modified meal, modified meal + resveratrol (5 or 20 mg) in social interaction and novelty test. Interaction time during session 1 (sec, A), interaction time during session 2 (sec, B), time spent (sec) in each chamber during session 1 (C) and time spent (sec) in each chamber during session 2 (D) in groups of control (first bar from left), modified meal (second bar), modified meal + resveratrol (5 or 20 mg, third and fourth bar). Error bars are represented as mean ± SEM, for two-way ANOVA, followed by Bonferroni's multiple comparison test. $^{a}p< 0.001$, $^{b}p<0.01$ and $^{c}p<0.001$ are the significance values

function markers (HbA1c, cholesterol, triglycerides, urea, creatinine, alanine aminotransferase, alkaline phosphatase, LDL, HDL), and preserved hippocampal integrity. These findings highlight resveratrol's neuroprotective potential against combined dietary and environmental stressors.

The behavioral analysis highlighted that the modified meal group caused significant memory impairments, consistent with previous findings. Resveratrol treatment improved long-term memory in the Morris water maze and short-term memory and decision-making in the Y-maze, indicating cognitive benefits. Social behavior also improved with resveratrol treatment. Overall, resveratrol improved learning and memory, consistent with previous findings (Wang et al., 2021). Moreover, resveratrol supplementation significantly improved metabolic dysfunction induced by the modified meal. Both 5 and 20 mg doses reduced elevated HbA1c levels, indicating improved glycemic control and supporting its anti-diabetic effects (Baur et al., 2006; Szkudelski and Szkudelska, 2011). It also reduces total cholesterol, triglycerides, and LDL, while increasing HDL. These changes suggest a cardio-metabolic protective role for resveratrol, likely via modulation of lipid metabolism, oxidative stress, and inflammatory pathways (Lagouge et al., 2006; Brasnyo et al., 2011).

In addition, the present study demonstrated that hepatic and renal functions were significantly impaired in the modified meal group. Resveratrol treatment, particularly at the 20 mg/kg dose, effectively attenuated these changes, significantly reducing alanine aminotransferase and creatinine levels and partially normalizing alkaline phosphatase, and bilirubin. The 5 mg/kg dose also showed beneficial effects, though the responses varied depending on the biomarker, suggesting a dose- and pathway-specific modulation. These outcomes highlight the hepatoprotective and nephron-protective properties of resveratrol, attributed to its antioxidant, anti-inflammatory, and enzymatic regulatory roles (Almeida et al., 2009; Lagouge et al., 2006; Zhang et al., 2021).

In parallel, histological assessments revealed significant neurodegeneration in hippocampal sub-regions (CA1, CA2, CA3, and dentate gyrus) in the modified meal group, indicating severe oxidative and metabolic stress. Resveratrol treatment markedly preserved neuronal integrity, with the 20 mg/kg dose showing near-complete restoration of neuronal density, consistent with its

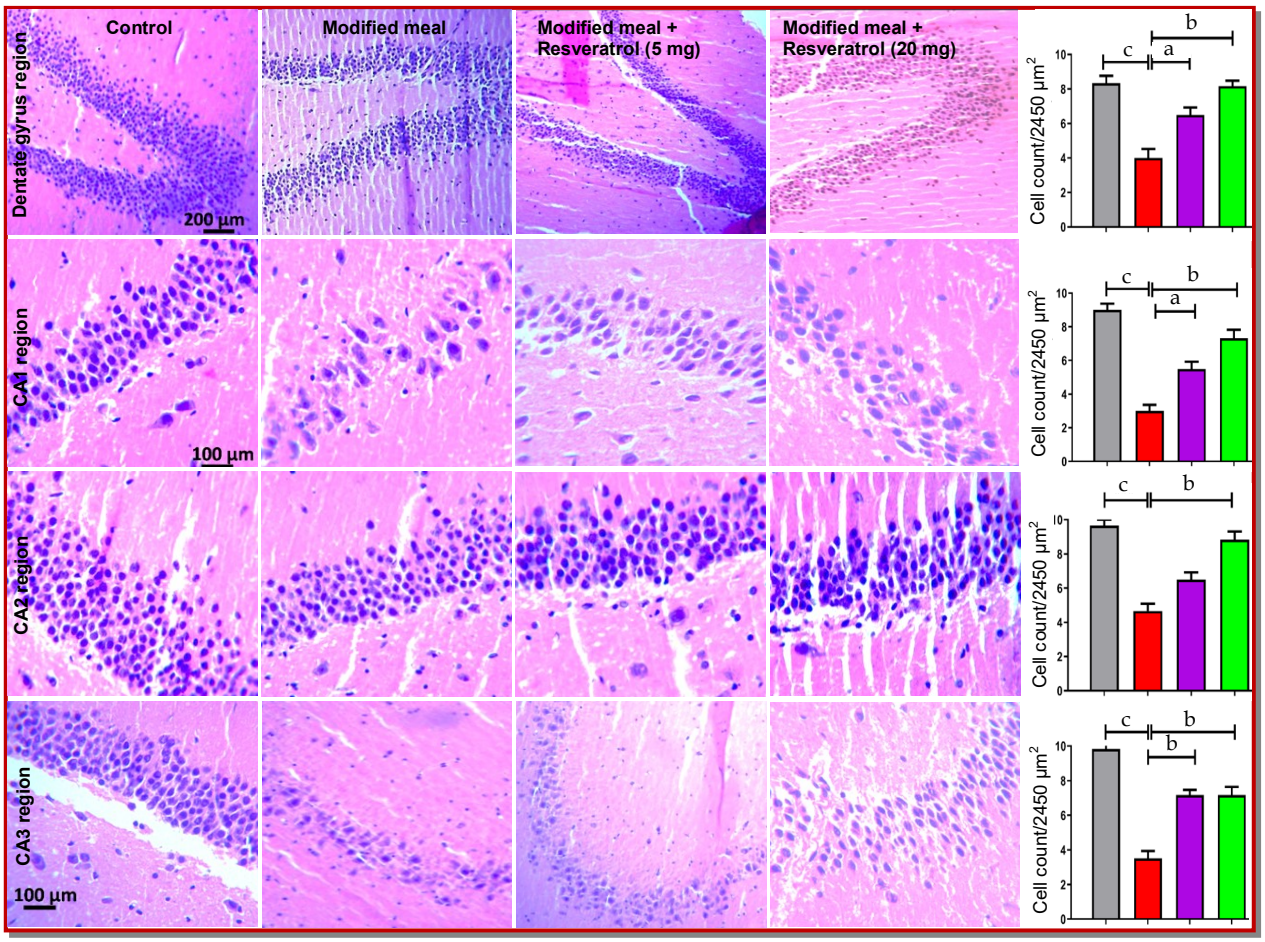

Figure 4: Histological pictures of dentate gyrus, CA1, CA2, and CA3 regions of hippocampus in control, modified meal, modified meal + resveratrol (5 or 20 mg). Right column shows the bar graphs of cell count in different groups. $^{a}p<0.01$, $^{b}p<0.001$ and $^{c}p<0.001$. (x40)

known neuroprotective mechanisms (Baur and Sinclair, 2006; Liu et al., 2017). Reducing neuronal loss supports resveratrol's potential to mitigate the cognitive and structural damage induced by environmental and dietary insults. These findings collectively highlight resveratrol's multifaceted protective role in preventing organ damage through its modulation of oxidative stress, inflammation, and cellular homeostasis.

These findings have important implications especially when considering global public health. Neurodevelopment and cognitive health are seriously threatened by the combined effects of high fat diet and metal exposure, particularly in areas with insufficient regulatory control. The findings from this study suggest that resveratrol (20 mg) could be an effective option to reverse cognitive deficits, histopathology, and changes in biochemical levels induced by modified meal exposure. However, more research is needed to translate these preclinical findings into practical practice.

Several pharmacological drugs and naturally occurring compounds have shown potential in preventing and treating cognitive and metabolic impairments. These include metformin, omega-3 fatty acids (DHA/EPA), donepezil (an acetylcholinesterase inhibitor), liraglutide (a GLP-1 receptor agonist), as well as natural compounds such as curcumin and berberine. These agents have been reported to enhance cognitive function in models of aging and metabolic syndrome (Du et al., 2022; Wang et al., 2012; Wei et al., 2023). In addition to their metabolic benefits, they exert antioxidant and anti-inflammatory effects and modulate neurotrophic signaling pathways, such as BDNF, thereby supporting synaptic plasticity and memory performance (Rainey-Smith et al., 2016; Sadraie et al., 2019). Resveratrol, a naturally occurring polyphenolic compound, has garnered attention for its diverse metabolic and neuroprotective benefits. Owing to its demonstrated antioxidant, anti-inflammatory, and cognitive-enhancing properties, it was selected for use in this study. It has been reported that resveratrol exerts neuroprotective effects by activating SIRT1 (sirtuin 1), a key regulatory protein involved in metabolism, cellular stress responses, and longevity. In the context of metal- induced neurotoxicity, resveratrol promotes hippocampal neurogenesis—the formation of new neurons in the hippocampus—by

Table I

Metabolic parameters in modified meal and resveratrol-treated groups

Variable	Control	Modified meal	Resveratrol (5 mg/kg)	Resveratrol (20 mg/kg)	p-value
HbA1c (%)	2.4 ± 0.3	4.1 ± 0.5	3.3 ± 0.3	3.0 ± 0.2	0.05
Total cholesterol (mmol/L)	69.1 ± 3.9	169.3 ± 27.8	84.1 ± 5.7	97.0 ± 11.2	0.01
Triglyceride (mmol/L)	144.7 ± 12.8	253.5 ± 15.3	135.8 ± 16.0	142.2 ± 7.6	0.001
High density lipoprotein (mg/dL)	41.6 ± 0.8	18.2 ± 0.9	35.5 ± 1.1	32.5 ± 0.7	0.001
Low density lipoprotein (mg/dL)	25.1 ± 1.7	61.8 ± 6.5	30.8 ± 3.4	38.8 ± 2.6	0.001
Alanine aminotransferase (U/L)	71.3 ± 0.8	112.0 ± 13.1	74.6 ± 6.4	62.0 ± 3.1	0.01
Alkaline phosphatase (U/L)	225.8 ± 3.8	660.7 ± 62.9	344.8 ± 37.7	554.8 ± 43.9	0.001
Total bilirubin (mg/dL)	0.4 ± 0.04	0.7 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.01
Urea (mg/dL)	21.0 ± 1.1	64.5 ± 5.8	31.6 ± 3.2	29.6 ± 3.4	0.001
Creatinine (mg/dL)	0.5 ± 0.1	1.38 ± 0.2	0.5 ± 0.03	0.4 ± 0.02	0.01

Values are expressed as mean ± SEM. n=6 animals in each group. Comparisons were made between control, modified meal (high fat diet and metal exposure), and resveratrol-treated groups (5 and 20 mg/kg). Statistical significance was assessed using one-way ANOVA followed by post hoc testing

modulating this signaling pathway. Activation of SIRT1 helps reduce oxidative stress and inflammation enhances mitochondrial function, and supports neuronal survival and plasticity, thereby counteracting the cognitive deficits associated with metal exposure (Wang et al., 2021). The SIRT1 activation leads to enhanced mitochondrial biogenesis which is crucial in both metabolic disorders (e.g., type 2 diabetes) and neurodegenerative diseases (e.g., Alzheimer's) (Price et al., 2012).

This study has shown promising effects of resveratrol, however, there are some limitations of the study. Expression analysis of additional protein markers can help better understand the underlying mechanism. Further investigations into the neuroinflammation markers in the human population can add more insights into this area.

Conclusion

This co-exposure to high fat diet and metals in drinking water is associated with abnormal liver enzyme and bilirubin levels, synaptic dysfunction, neuroinflammation, and hippocampal memory impairments. Resveratrol, shows promise in targeting these pathways, making it a potential therapy for neurodegeneration, metabolic disorders, and cognitive decline.

Financial Support

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Ethical Issue

All the performed experiments were following the rulings of the Institute of Laboratory Animal Research, Division on Earth and Life Sciences, National Institute of Health, USA (Guide for the Care and Use of Laboratory Animals). Every protocol used in this research was approved by the Institutional Review Board (IRB), Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad (IRB-135).

Conflict of Interest

Authors declare no conflict of interest.

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References

Alarcon De La Lastra C, Villegas I. Resveratrol as an anti-inflammatory and anti-aging agent: Mechanisms and clinical implications. Mol Nutr Food Res. 2005; 49: 405-30.

Almeida L, Vaz-da-Silva M, Falcao A, Soares E, Costa R, Loureiro AI, Fernandes-Lopes C, Rocha JF, Nunes T, Wright L, Soares-da-Silva P. Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers. Mol Nutr Food Res. 2009; 53: S7-S15.

Alwan, A. Global status report on noncommunicable diseases 2010. World Health Organization. Geneva, 2011.

Baile CA, Yang JY, Rayalam S, Hartzell DL, Lai CY, Andersen C, Della-Fera MA. Effect of resveratrol on fat mobilization. Ann N Y Acad Sci. 2011; 1215: 40-7.

Bastianetto S, Ménard C, Quirion R. Neuroprotective action of resveratrol. Biochim Biophys Acta Mol Basis Dis. 2015; 1852: 1195-201.

Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006; 444: 337-42.

Baur JA, Sinclair DA. Therapeutic potential of resveratrol: The *in vivo* evidence. Nat Rev Drug Discov. 2006; 5: 493-506.

Bjørklund G, Skalny AV, Rahman MM, Dadar M, Yassa HA, Aaseth J, Chirumbolo S, Skalnaya MG, Tinkov AA. Toxic metal(loid)-based pollutants and their possible role in autism spectrum disorder. Environ Res. 2018; 166: 234-50.

Boitard C, Cavaroc A, Sauvant J, Aubert A, Castanon N, Laye S, Ferreira G. Impairment of hippocampal-dependent memory induced by juvenile high fat diet intake is associated with enhanced hippocampal inflammation in rats. Brain Behav Immun. 2014; 40: 9-17.

Brasnyo P, Molnar GA, Mohas M, Marko L, Laczy B, Cseh J, Mikolas E, Szijarto IA, Merei A, Halmai R, Meszaros LG, Sümegi B, Wittmann I. Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. Br J Nutr. 2011; 106: 383-89.

Briffa J, Sinagra E, Blundell R. Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon 2020; 6: e05047.

Cathe DS, Whitaker JN, Breitner EK, Comfort KK. Exposure to metal oxide nanoparticles in physiological fluid induced synergistic biological effects in a keratinocyte model. Toxicol Lett. 2017; 268: 1-7.

Clemente-Suárez VJ, Beltrán-Velasco AI, Redondo-Flórez L, Martín-Rodríguez A, Tornero-Aguilera JF. Global impacts of western diet and its effects on metabolism and health: A narrative review. Nutrients 2023; 15: 2749.

Du H, Meng X, Yao Y, Xu J. The mechanism and efficacy of GLP-1 receptor agonists in the treatment of Alzheimer's disease. Front Endocrinol. 2022; 13: 1033479.

Francis H, Stevenson R. The longer-term impacts of western diet on human cognition and the brain. Appetite 2013; 63: 119-28.

Freire C, Amaya E, Gil F, Fernández MF, Murcia M, Llop S, Andiarena A, Aurrekoetxea J, Bustamante M, Guxens M, Ezama E, Fernandez-Tardón G, Olea N. Prenatal co-exposure to neurotoxic metals and neurodevelopment in preschool children: The Environment and Childhood (INMA) project. Sci Total Environ. 2018; 621: 340-51.

Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome: An American Heart Association/ National Heart, Lung, and Blood Institute scientific statement. Circulation 2005; 112: 2735-52.

Imamura F, Micha R, Wu JHY, de Oliveira Otto MC, Otite FO, Abioye AI, Mozaffarian D. Effects of saturated fat, polyunsaturated fat, monounsaturated fat, and carbohydrate on glucose-insulin homeostasis: A systematic review and meta-analysis of randomised controlled feeding trials. PLoS Med. 2016; 13: e1002087.

Iqbal G, Ahmed T. Co-exposure of metals and high fat diet causes aging-like neuropathological changes in non-aged mice brain. Brain Res Bull. 2019; 147: 148-58.

Iqbal G, Iqbal G, Iqbal A, Mahboob A, Farhat SM, Ahmed T. Memory enhancing effect of black pepper in the $AlCl_3$ induced neurotoxicity mouse model is mediated through its active component chavicine. Curr Pharm Biotechnol. 2016; 17: 962-73.

Iqbal G, Zada W, Mannan A, Ahmed T. Elevated heavy metals levels in cognitively impaired patients from Pakistan. Environ Toxicol Pharmacol. 2018; 60: 100-09.

Ishaq S, Zahid S, Ahmed T. Sex-specific effects of neuromodulatory drugs on normal and stress-induced social dominance and aggression in rats. Psychopharmacology (Berl). 2023; 242: 1011-24.

Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating Sirt1 and PGC-1α. Cell 2006; 127: 1109-22.

Lamas GA, Navas-Acien A, Mark DB, Lee KL. Heavy metals, cardiovascular disease, and the unexpected benefits of chelation therapy. J Am Coll Cardiol. 2016; 67: 2411-18.

Liu T, Liang X, Lei C, Huang Q, Song W, Fang R, Li C, Li X, Mo H, Sun N, Lv H, Liu Z. High fat diet affects heavy metal accumulation and toxicity to mice liver and kidney probably via gut microbiota. Front Microbiol. 2020; 11: 1604.

Liu W, Chen P, Deng J, Lv J, Liu J. Resveratrol and polydatin as modulators of Ca^{2+} mobilization in the cardiovascular system. Ann N Y Acad Sci. 2017; 1403: 82-91.

López E, Arce C, Oset-Gasque MJ, Cañadas S, González MP. Cadmium induces reactive oxygen species generation and lipid peroxidation in cortical neurons in culture. Free Radic Biol Med. 2006; 40: 940-51.

Mazzocco JC, Jagadapillai R, Gozal E, Kong M, Xu Q, Barnes GN, Freedman JH. Disruption of essential metal homeostasis in the brain by cadmium and high fat diet. Toxicol Rep. 2020; 7: 1164-69.

Meng T, Xiao D, Muhammed A, Deng J, Chen L, He J. Anti-inflammatory action and mechanisms of resveratrol. Molecules 2021; 26: 229.

Mitra S, Chakraborty AJ, Tareq AM, Emran TB, Nainu F, Khusro A, Idris AM, Khandaker MU, Osman H, Alhumaydhi FA, Simal-Gandara J. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. J King Saud Univ Sci. 2022; 34: 101865.

Morris MC. The role of nutrition in Alzheimer's disease: Epidemiological evidence. Eur J Neurol. 2009; 16(Suppl 1): 1 -7.

Morrison CD, Pistell PJ, Ingram DK, Johnson WD, Liu Y, Fernandez-Kim SO, White CL, Purpera MN, Uranga RM, Bruce-Keller AJ, Keller JN. High fat diet increases hippocampal oxidative stress and cognitive impairment in aged mice: Implications for decreased Nrf2 signaling. J Neurochem. 2010; 114: 1581-89.

Park SS, Skaar DA, Jirtle RL, Hoyo C. Epigenetics, obesity and early-life cadmium or lead exposure. Epigenomics 2017; 9: 57-75.

Pistell PJ, Morrison CD, Gupta S, Knight AG, Keller JN, Ingram DK, Bruce-Keller AJ. Cognitive impairment following high fat diet consumption is associated with brain inflammation. J Neuroimmunol. 2010; 219: 25-32.

Price NL, Gomes AP, Ling AJ, Duarte FV, Martin-Montalvo A, North BJ, Agarwal B, Ye L, Ramadori G, Teodoro JS. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab. 2012; 15: 675-90.

Qiao Y, Sun J, Xia S, Tang X, Shi Y, Le G. Effects of resveratrol on gut microbiota and fat storage in a mouse model with high fat-induced obesity. Food Funct. 2014; 5: 1241-49.

Raehsler SL, Choung RS, Marietta EV, Murray JA. Accumulation of heavy metals in people on a gluten-free diet. Clin Gastroenterol Hepatol. 2018; 16: 244-51.

Rainey-Smith SR, Brown BM, Sohrabi HR, Shah T, Goozee KG, Gupta VB, Martins RN. Curcumin and cognition: A randomised, placebo-controlled, double-blind study of community-dwelling older adults. Br J Nutr. 2016; 115: 2106-13.

Rivera L, Moron R, Zarzuelo A, Galisteo M. Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. Biochem Pharmacol. 2009; 77: 1053-63.

Rotter I, Kosik-Bogacka D, Dołęgowska B, Safranow K, Lubkowska A, Laszczynska M. Relationship between the concentrations of heavy metals and bioelements in aging men with metabolic syndrome. Int J Environ Res Public Health. 2015; 12: 3944-61.

Sadraie S, Kiasalari Z, Razavian M, Azimi S, Sedighnejad L, Afshin-Majd S, Baluchnejadmojarad T, Roghani M. Berberine ameliorates lipopolysaccharide-induced learning and memory deficit in the rat: Insights into underlying molecular mechanisms. Metab Brain Dis. 2019; 34: 245-55.

Shaibah HS, Elsify AK, Medhat TM, Rezk HM, El-Sherbiny M. Histopathological and immunohistochemical study of the protective effect of triptorelin on the neurocytes of the hippocampus and the cerebral cortex of male albino rats after short-term exposure to cyclophosphamide. J Microsc Ultrastruct. 2016; 4: 123-32.

Shukla A, Shukla GS, Srimal RC. Cadmium-induced alterations in blood-brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat. Hum Exp Toxicol. 1996; 15: 400-05.

Szkudelski T, Szkudelska K. Anti-diabetic effects of resveratrol. Ann N Y Acad Sci. 2011; 1215: 34-39.

Tan BL, Norhaizan ME. Effect of high fat diets on oxidative stress, cellular inflammatory response, and cognitive function. Nutrients 2019; 11: 2579.

Wali JA, Jarzebska N, Raubenheimer D, Simpson SJ, Rodionov RN, O'Sullivan JF. Cardio-metabolic effects of high fat diets and their underlying mechanisms: A narrative review. Nutrients 2020; 12: 1505.

Wang J, Gallagher D, DeVito LM, Cancino GI, Tsui D, He L, Keller GM, Frankland PW, Kaplan DR, Miller FD. Metformin activates an atypical PKC-CBP pathway to promote neurogenesis and enhance spatial memory formation. Cell Stem Cell. 2012; 11:23-35.

Wang R, Wu Z, Bai L, Liu R, Ba Y, Zhang H, Cheng X, Zhou G, Huang H. Resveratrol improved hippocampal neurogenesis following lead exposure in rats through activation of SIRT1 signaling. Environ Toxicol. 2021; 36: 1664-73.

Wang X, Han X, Guo S, Ma Y, Zhang Y. Associations between patterns of blood heavy metal exposure and health outcomes: Insights from NHANES 2011–2016. BMC Public Health. 2024; 24: 558.

Wang X, Mukherjee B, Park SK. Associations of cumulative exposure to heavy metal mixtures with obesity and its comorbidities among US adults in NHANES 2003–2014. Environ Int. 2018; 121: 683-94.

Wei BZ, Li L, Dong CW, Tan CC, for the Alzheimer's Disease Neuroimaging Initiative, Xu W. The relationship of omega-3 fatty acids with dementia and cognitive decline: evidence from prospective cohort studies of supplementation, dietary intake, and blood markers. Am J Clin Nutr. 2023; 117: 1096-109.

Wu KG, Chang CY, Yen CY, Lai CC. Associations between environmental heavy metal exposure and childhood asthma: A population-based study. J Microbiol Immunol Infect. 2019; 52: 352-62.

Zahoor M, Farhat SM, Khan S, Ahmed T. Daidzin improves neurobehavioral outcome in rat model of traumatic brain injury. Behav Brain Res. 2024; 472: 115158.

Zhang LX, Li CX, Kakar MU, Khan MS, Wu PF, Amir RM, Dai DF, Naveed M, Li QY, Saeed M, Shen JQ, Rajput SA, Li JH. Resveratrol (Rv): A pharmacological review and call for further research. Biomed Pharmacother. 2021; 143: 112164.

Zhang LX, Li CX, Kakar MU, Khan MS, Wu PF, Amir RM, Dai DF, Naveed M, Li QY, Saeed M, Shen JQ. Resveratrol (Rv): A pharmacological review and call for further research. Biomed Pharmacother. 2021; 143: 112164.

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