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Dietary Buckwheat and Bisphenol Exposure: Tissue-Specific Sirt1 Responses and Implications For Food Safety Regulation

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ABSTRACT

Culinary micro, small, and medium enterprises (MSMEs) in West Java operate in a Background: Bisphenol A (BPA) is an endocrine-disrupting chemical associated with oxidative stress, metabolic disturbances, and alterations in cellular stress-response pathways. Sirtuin 1 (SIRT1), an NAD^+-dependent protein deacetylase, plays an important role in regulating cellular metabolism, stress adaptation, and inflammatory responses. Buckwheat (Fagopyrum esculentum Moench) contains several biologically active flavonoids that may influence these processes.

Objective: This study investigated the effects of a whole-grain buckwheat diet on tissue-specific SIRT1 protein levels in mice exposed to BPA and characterized the major flavonoids present in the dietary material.

Methods: Thirty-two male mice were randomly allocated to four experimental groups (n = 8 per group): control, buckwheat diet, BPA exposure, and combined buckwheat diet and BPA exposure. The dietary intervention consisted of a 50:50 (w/w) mixture of whole and ground buckwheat seeds provided as the sole food source for 25 days. BPA was administered through drinking water at a concentration of 15 mg/L. SIRT1 protein concentrations in liver, kidney, and stomach tissue homogenates were determined using a mouse-specific ELISA. The phytochemical composition of buckwheat extracts was investigated using HPLC and LC–MS, while molecular docking was performed to evaluate the potential interactions of selected flavonoids with SIRT1.

Results: SIRT1 protein concentrations exhibited distinct tissue-dependent patterns across the experimental groups. In the liver, the mean SIRT1 concentration was 4.34 ng/mL in controls, 3.73 ng/mL following BPA exposure, and 4.67 ng/mL in the combined-treatment group. Corresponding kidney values were 5.40, 4.65, and 5.91 ng/mL, respectively. In contrast, gastric SIRT1 concentrations were higher in the BPA group (2.56 ng/mL) than in controls (2.39 ng/mL), whereas the combined-treatment group showed a lower mean value (2.15 ng/mL). HPLC analysis identified rutin (1.032 ± 0.221 mg/g), dihydroquercetin (0.404 ± 0.102 mg/g), and quercetin (0.308 ± 0.086 mg/g) as major quantified flavonoids. Molecular docking predicted binding affinities of −10.4 kcal/mol for rutin and −9.5 kcal/mol for quercetin toward the selected SIRT1 structure.

Conclusion: The findings indicate that a whole-grain buckwheat diet is associated with tissue-specific differences in SIRT1 protein responses during BPA exposure. The observed patterns suggest a potential modulatory role of buckwheat dietary constituents; however, the contribution of individual flavonoids and the underlying molecular mechanisms require further experimental investigation.

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1. Introduction

Bisphenol A (BPA) is an industrial chemical widely used in the manufacture of polycarbonate plastics and epoxy resins, resulting in its widespread occurrence in food-contact materials and other consumer products. Human and experimental exposure to BPA has raised considerable concerns because of its endocrine-disrupting properties and potential effects on multiple physiological systems. Experimental studies have associated BPA exposure with oxidative stress, mitochondrial dysfunction, inflammatory responses, and disturbances in cellular metabolic regulation [5, 22, 26]. These effects are particularly relevant to metabolically active organs, including the liver and kidneys, which contribute substantially to xenobiotic metabolism, detoxification, and the elimination of potentially harmful compounds. The gastrointestinal tract may also be affected by BPA exposure through local interactions with epithelial cells, inflammatory pathways, and the intestinal microbiota [26]. Nevertheless, the magnitude and direction of BPA-associated molecular responses may differ considerably among tissues, indicating that systemic exposure does not necessarily produce uniform biological effects. Sirtuin 1 (SIRT1) is an NAD^+-dependent protein deacetylase involved in the regulation of cellular metabolism, oxidative stress responses, inflammation, mitochondrial function, and genomic stability. Through interactions with several transcriptional regulators and signaling proteins, including PGC-1α, FOXO, and NF-κB, SIRT1 contributes to the adaptation of cells to metabolic and environmental stress [27, 28]. Its biological functions are closely associated with intracellular NAD^+ availability, which links cellular energy metabolism to stress-response regulation [3, 13]. Alterations in SIRT1 expression and activity have been reported in experimental models of toxicant exposure and metabolic dysfunction. In particular, BPA-associated changes in SIRT1-related pathways have been documented in experimental animals and cellular models, suggesting that this protein may be involved in the response to endocrine-disrupting chemicals [5, 22]. However, changes in SIRT1 protein abundance should be distinguished from changes in its enzymatic activity, since these parameters do not necessarily vary in parallel. Therefore, evaluating tissue-specific SIRT1 protein levels provides a useful, although incomplete, indication of molecular responses to environmental stressors. Dietary plant-derived bioactive compounds have attracted increasing attention as potential modulators of cellular stress-response pathways. Common buckwheat (*Fagopyrum esculentum* Moench) is a nutritionally valuable pseudocereal containing flavonoids and phenolic acids, including rutin, quercetin, dihydroquercetin, kaempferol derivatives, vitexin, isoorientin, chlorogenic acid, and caffeic acid [6, 12, 14, 23]. The composition and abundance of these compounds depend on genetic characteristics, growing conditions, plant tissues, and processing methods [14, 23]. Rutin and quercetin, in particular, have been investigated for their antioxidant and anti-inflammatory properties and their potential involvement in signaling pathways associated with cellular metabolic regulation [7, 8]. Experimental evidence also suggests that quercetin and rutin may attenuate tissue damage associated with exposure to BPA and other potentially toxic compounds [15, 19, 21]. Furthermore, studies involving buckwheat-enriched diets have reported beneficial changes in metabolic and inflammatory parameters, including alterations in the PGC-1α/SIRT1 signaling axis in experimental models [11, 20]. These observations provide a rationale for investigating whole-grain buckwheat as a dietary intervention under conditions of chemical stress. Importantly, the biological effects of a whole-food intervention cannot be attributed exclusively to individual flavonoids because nutritional composition, dietary energy intake, and interactions among multiple food constituents may also contribute to the observed responses. Despite growing interest in the biological properties of buckwheat and the regulatory functions of SIRT1, the tissue-specific effects of a whole-grain buckwheat diet during BPA exposure remain insufficiently characterized. Previous investigations have frequently focused on isolated flavonoids, concentrated plant extracts, or specific metabolic outcomes rather than comparing SIRT1 protein responses across several organs following a dietary intervention based on whole buckwheat seeds. In addition, the potential molecular interactions between major buckwheat flavonoids and SIRT1 provide an interesting complementary research direction, although computational predictions alone cannot establish direct enzyme activation or explain in vivo biological effects [7, 17]. Addressing these questions may help clarify whether the response to a buckwheat-based diet differs between organs exposed to the same chemical stressor.

Accordingly, the present study aimed to investigate the effects of a whole-grain buckwheat diet, consisting of a 50:50 mixture of whole and ground *F. esculentum* seeds, on SIRT1 protein concentrations in the liver, kidneys, and stomach of male mice under basal conditions and during experimental BPA exposure. The study additionally characterized selected flavonoids in buckwheat extracts using HPLC and LC–MS and evaluated the predicted interactions of rutin and quercetin with the SIRT1 protein structure through molecular docking. By integrating tissue-level protein measurements, phytochemical characterization, and computational analysis, this investigation sought to provide a more comprehensive assessment of the potential relationship between whole-grain buckwheat consumption and tissue-specific SIRT1 responses to BPA exposure.

2. Materials and Methods

2.1. Experimental Animals and Ethical Approval

The experimental study was conducted using 32 male albino mice (*Mus musculus*), aged 8 weeks and weighing 20–25 g at the

beginning of the experiment. The animals were allocated into four experimental groups, with eight animals in each group (n = 8). Before the experiment, the mice were allowed to acclimatize to the laboratory environment for one week. The animals were housed under controlled environmental conditions, including a temperature of 22 ± 2°C, relative humidity of 55 ± 5%, and a 12-h light/12-h dark cycle. Standard laboratory conditions were maintained throughout the experimental period. All experimental procedures were performed in accordance with relevant institutional and international guidelines for the care and use of laboratory animals. The experimental protocol was approved by the Bioorganic Chemistry Ethics Committee (approval number: № 1-21451-2-524, date: 31.10.2025).

2.2. Experimental Design and Treatment Groups

The experimental period lasted 25 days. The animals were randomly assigned to four groups (n = 8 per group):

- Group I – Control: mice received standard laboratory chow and plain drinking water.
- Group II – Buckwheat: mice received a buckwheat-based diet consisting of a 50:50 (w/w) mixture of whole and ground *Fagopyrum esculentum* seeds, with plain drinking water.
- Group III – BPA: mice received standard laboratory chow and drinking water containing bisphenol A (BPA) at a concentration of 15 mg/L.
- Group IV – BPA + Buckwheat: mice received the same buckwheat-based diet as Group II and drinking water containing BPA at 15 mg/L.

The buckwheat-based diet was provided as the sole food source in Groups II and IV during the experimental period. The drinking solutions and food were replenished every 2–3 days. Body weight was recorded weekly. The study was designed to investigate changes in tissue-specific SIRT1 protein levels following BPA exposure and the administration of a whole-grain buckwheat diet.

2.3. Preparation and Administration of Bisphenol A

Bisphenol A (BPA; purity ≥99%; Sigma-Aldrich, USA) was used to induce experimental exposure. A stock solution was prepared by dissolving BPA in 1 mL of 96% ethanol to obtain a concentration of 15 mg/mL. The stock solution was subsequently diluted in drinking water to achieve a final BPA concentration of 15 mg/L. The BPA-containing drinking solution was freshly prepared every 2–3 days and supplied to the animals in Groups III and IV throughout the 25-day experimental period. The control and buckwheat-only groups received plain drinking water. The nominal BPA concentration in drinking water was 15 mg/L. Individual BPA intake was not directly quantified because individual water consumption was not recorded. Therefore, the administered concentration is reported as the drinking-water concentration rather than as a confirmed individual daily dose in mg/kg body weight.

2.4. Buckwheat Material and Dietary Preparation

Common buckwheat (*Fagopyrum esculentum* Moench) seeds were used in the dietary intervention. The seeds were prepared as a mixture of whole and ground seeds in a 1:1 weight ratio (50:50, w/w). This mixture was provided as the sole food source to animals assigned to the buckwheat-containing groups. The dietary intervention was designed to assess the overall effects of a whole-grain buckwheat-based diet. Since the intervention involved the complete seed material rather than isolated flavonoids, the observed effects were interpreted in the context of the combined nutritional and phytochemical composition of buckwheat.

2.5. Tissue Collection and Preparation of Homogenates

At the end of the 25-day experimental period, the mice were anesthetized by exposure to isoflurane vapor generated from isoflurane-soaked cotton material. Following loss of consciousness, euthanasia was performed by decapitation. Liver, kidney, and stomach tissues were immediately collected for subsequent biochemical analyses. The collected tissues were washed with ice-cold phosphate-buffered solution to remove residual blood and homogenized in phosphate buffer supplemented with protease inhibitors. The homogenates were centrifuged at 10,000 × g for 10 min at 4°C. The resulting supernatants were collected and used for the determination of SIRT1 protein levels. All tissue samples were maintained under appropriate cold-storage conditions (-80°C) until biochemical analysis.

2.6. Determination of SIRT1 Protein Levels by ELISA

SIRT1 protein levels in liver, kidney, and stomach tissue homogenates were determined using mouse-specific enzyme-linked immunosorbent assay (ELISA) kits (CUSABIO, China), according to the manufacturer's instructions. Briefly, tissue homogenate supernatants were added to the designated microplate wells, followed by the assay reagents supplied with the kit. After the prescribed incubation and washing steps, the colorimetric reaction was developed using the substrate solution and stopped with the stop reagent. Absorbance was measured using a microplate reader at the wavelength specified by the manufacturer. The SIRT1 concentrations were

calculated from the standard calibration curve provided with the assay. Each sample was analyzed in duplicate, and the mean of the duplicate measurements was used for subsequent statistical analysis. The following kit information should be reported to ensure reproducibility: Elabscience Biotechnology Inc., China; Cat. No. E-EL-M0350; Lot No. WA066624P5202; 48 tests. The final unit of SIRT1 measurement should also be stated consistently. If concentrations were normalized to total protein content, the protein assay method and normalization procedure must be described.

2.7. Extraction of Buckwheat Phenolic Compounds

For phytochemical analysis, buckwheat seed samples were extracted using aqueous ethanol. Briefly, 5–10 g of the prepared seed material was mixed with 50 mL of 70% ethanol and extracted for 2 h. The sample was subsequently re-extracted twice using 25 mL portions of the same solvent. The extracts were combined and adjusted to a final volume of 100 mL. The resulting mixture was centrifuged at 6,000–8,000 rpm for 20–30 min to remove suspended particles. The clarified supernatant was collected for chromatographic analysis.

2.8. HPLC Analysis of Selected Flavonoids

The concentrations of selected phenolic compounds in the buckwheat extract were determined using a high-performance liquid chromatography (HPLC) system (Agilent 1200 series, Agilent Technologies, USA).

Chromatographic separation was performed using an Eclipse XDB-C18 column (5 μm, 4.6 × 250 mm). The column temperature was maintained at 30°C. The mobile phase consisted of phosphate buffer and acetonitrile, delivered at a flow rate of 0.8 mL/min. The injection volume was 10 μL. Detection was performed using a diode-array detector (DAD) at 254 and 272 nm. The gradient elution program was as follows: 95:5 (phosphate buffer) from 0 to 5 min; 70:30 from 6 to 12 min; 50:50 from 12 to 13 min; and 95:5 from 13 to 15 min. Rutin, quercetin, and dihydroquercetin were identified by comparison of their retention times and UV absorption characteristics with those of corresponding reference standards. Their concentrations were calculated using external calibration curves prepared from the respective standards and expressed as mg/g of buckwheat material.

2.9. LC–MS-Based Phytochemical Characterization

Liquid chromatography–mass spectrometry (LC–MS) was additionally used to characterize the phytochemical constituents of the buckwheat extract. Analyses were reportedly performed using electrospray ionization (ESI) in positive and negative ionization modes.

The LC–MS method requires further technical details before this subsection can be considered reproducible. The following information must be added from the original instrument records: the LC–MS instrument model, chromatographic column, mobile-phase composition, gradient program, flow rate, injection volume, column temperature, ionization source parameters, capillary voltage, gas temperature and flow, mass acquisition range, scan mode, and collision energy (if MS/MS was performed). Compound assignments should be based on accurate mass measurements and, where available, fragmentation patterns and comparison with authentic reference standards or reliable spectral databases. Tentative identifications should be explicitly distinguished from compounds confirmed using reference standards. Compounds for which adequate mass-spectral evidence is unavailable and should not be reported as definitively identified.

2.10. Molecular Docking Analysis

Molecular docking was performed to investigate the predicted interactions of selected buckwheat flavonoids with the SIRT1 protein. The three-dimensional structures of rutin (SANC01085) and quercetin (SANC00317) were used as ligands. The crystal structure of SIRT1 (PDB ID: 4I5I) was retrieved from the Protein Data Bank. Docking calculations were conducted using the CB-Dock2 platform. The binding pocket was identified, and the docking search region was defined using the coordinates reported for the selected protein structure (center: x = 42.892, y = −27.350, z = 18.132). The docking search-space dimensions were selected according to the identified binding cavity. The predicted binding energies and ligand–protein interactions were evaluated to compare the relative binding poses of rutin and quercetin within the selected SIRT1 structure. Amino acid residues involved in the predicted interactions were recorded and examined to characterize the potential binding modes. Docking results were interpreted as computational predictions of possible molecular interactions. They were not considered direct evidence of SIRT1 activation, enzymatic activity, or a causal mechanism underlying the observed changes in tissue SIRT1 protein levels.

2.11. Statistical Analysis

Quantitative data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism version 8 (GraphPad Software, USA). The original analysis used one-way analysis of variance (ANOVA), followed by

Tukey's multiple-comparison test. Statistical significance was defined as $p < 0.05$. Because the experimental design included two factors, BPA exposure and dietary intervention, a two-way factorial ANOVA is also appropriate for evaluating the main effects of BPA exposure and buckwheat diet, as well as their interaction. This analysis should be performed using the individual animal-level data, with the tissue-specific outcomes analyzed separately. The final manuscript should report the statistical model actually applied, the relevant test statistics, degrees of freedom, exact p-values, and the number of biological replicates.

3. Results

Phytochemical composition of buckwheat extracts High-performance liquid chromatography (HPLC) analysis of 70% ethanolic buckwheat extracts identified three major flavonoids. Rutin was the most abundant compound (1.032 ± 0.221 mg/g), followed by dihydroquercetin (0.404 ± 0.102 mg/g) and quercetin (0.308 ± 0.086 mg/g).

Table 1. Concentration of major flavonoids identified in buckwheat extract using HPLC analysis

Flavonoids name	Concentration mg/gr
rutin	1.032±0.221
quercetin	0.308±0.086
dihydroquercetin	0.404±0.102

Liquid chromatography–mass spectrometry (LC-MS) analysis in positive and negative ESI modes confirmed the presence of rutin (quercetin-3-rutinoside, m/z 611.35), quercetin, kaempferol-3-O-rutinoside (m/z 525.18), isoorientin (m/z 381.14) and vitexin (m/z 385.26), chlorogenic acid (m/z 354.08/360.22), caffeic acid (m/z 158.20), and quinic acid (m/z 192.12). Rutin was the predominant flavonoid in the ethanolic extract.

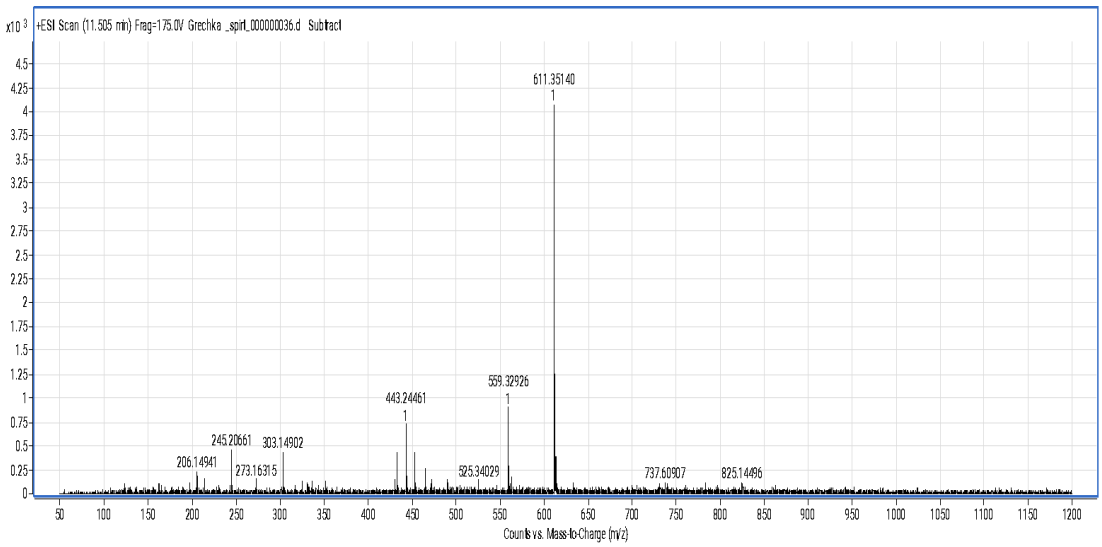


Figure 1. LC–MS spectrum of the ethanolic extract of buckwheat

Mass spectrometry analysis of the aqueous extract identified several metabolites based on their m/z (mass-to-charge ratio) values. The most prominent peak was attributed to cyanidin-related compounds with an m/z value near 350–400, confirming the presence of anthocyanins. In addition, succinic acid and ribose arsenate were detected with m/z values approximately at 130 and 250, respectively.

Table 2. : Major phytochemicals identified in buckwheat extracts by LC–MS analysis.

Compounds name	m/z	Chemical class	Extract type
Rutin (quercetin 3 rutinoside)	611.35	Flavonoid	Ethanolic extract
Kaempferol-3-O-rutinoside	—	Flavonoid glycoside	Ethanolic extract
Isoorientin / Vitexin	525.18	C-glycosyl flavonoid	Ethanolic extract
Chlorogenic acid	381.14 / 385.26	Phenolic acid	both ethanolic and aqueous

Caffeic acid	354.08 / 360.22	Phenolic acid	both ethanolic and aqueous
Quinic acid	158.20	Organic acid	both ethanolic and aqueous
Rutin (quercetin-3-O-rutinoside)	192.12	Flavonoid glycoside	Aqueous extract

Table 2 summarizes compound name, detected m/z values, chemical class, and the type of extract in which each compound was observed (ethanolic and/or aqueous extract). The results confirm that buckwheat extracts are rich in flavonoids (particularly rutin and other flavonoid glycosides) as well as phenolic and organic acids. Further MS analysis of the ethanolic extract revealed a wider range of bioactive compounds. The identified molecules included resveratrol (m/z ~470), diglycerides (m/z ~620), and phosphorylated ribose derivatives such as 5-phospho-α-D-ribose 1-diphosphate (m/z ~380).

Tissue SIRT1 protein levels

Table 3. SIRT1 protein levels in across experimental groups.

Samples	Control (A)	Buckwheat (B)	BPA (C)	Buckwheat+BPA (D)
Blood	1,541±0,15	1,97±0,11	1,70±0,09	1,93±0,11
Liver	4,342±0,21	3,94±0,08	3,73±0,10	4,67±0,19
Stomach	2,39±0,10	2,172±0,148	2,56±0,15	2,15±0,10
Kidney	5,40±0,04	4,73±0,16	4,65±0,138	5,91±0,10

Data are expressed as mean ± SEM (n = 8 per group) and presented as ng/mL tissue protein. Control: standard chow + plain water; Buckwheat: exclusive 50:50 whole and ground buckwheat diet; BPA: standard chow + 15 mg/L bisphenol A in drinking water; Buckwheat + BPA: exclusive buckwheat diet + 15 mg/L BPA. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test. Differences were considered statistically significant at $p < 0.05$.

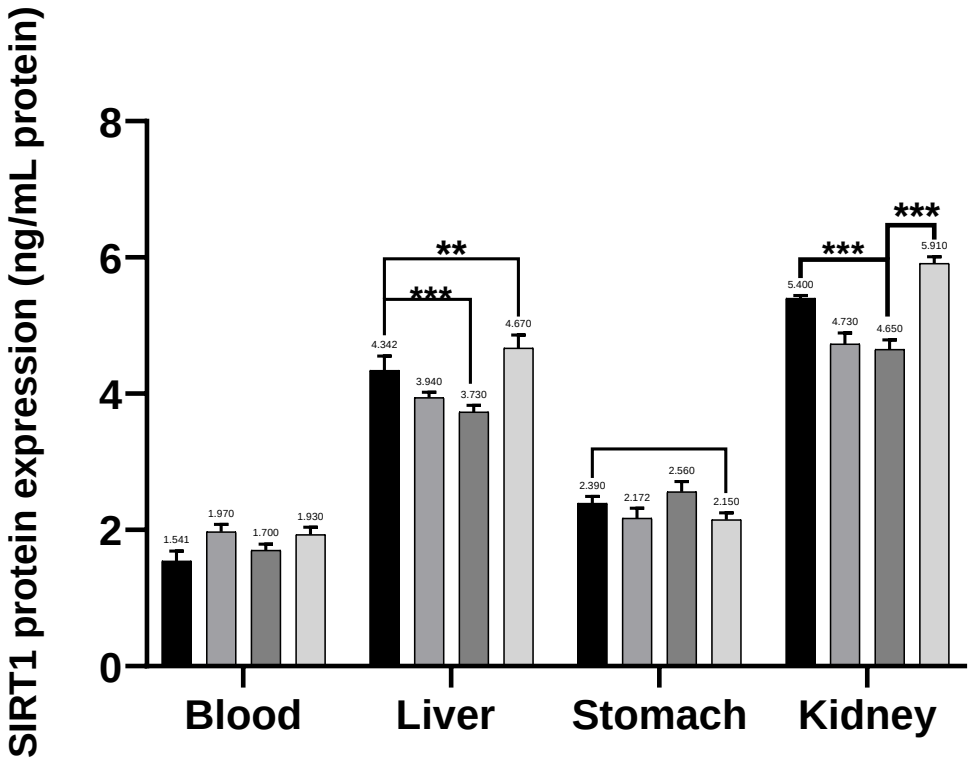


Figure 2. SIRT1 protein levels (ng/mg tissue lysate) in different tissues across experimental groups
Bars represent mean ± SEM. Control group (black), buckwheat diet only (gray), BPA only (light gray), buckwheat + BPA (dark gray).

In liver and kidney, BPA significantly decreased SIRT1 levels (***$p < 0.001$), while co-administration of buckwheat not only

restored but also elevated SIRT1 above control values (**p < 0.01 and ***p < 0.001); In the stomach, BPA induced a modest increase, which was normalized or slightly reduced by buckwheat diets; no significant differences were observed in blood. These results demonstrate tissue-specific modulation of SIRT1 by the buckwheat diet, with strong protective restoration against BPA-induced depletion in metabolically active tissues (liver and kidney).

In liver and kidney tissues, the exclusive buckwheat diet alone modestly decreased SIRT1 protein levels compared to the control group. Exposure to BPA alone resulted in a significant reduction in SIRT1 content. However, co-administration of buckwheat with BPA not only significantly restored SIRT1 levels relative to the BPA-only group but also elevated them above control values (liver: +25.2%; kidney: +9.4% relative to control). In contrast, stomach tissue exhibited a distinct response pattern: BPA exposure alone increased SIRT1 levels (+7.1% compared to control), whereas both the buckwheat-only and BPA + buckwheat groups showed values close to or slightly below control levels. One-way ANOVA followed by Tukey's post-hoc test confirmed statistically significant differences among groups ($p < 0.05$ for relevant comparisons). These findings demonstrate clear tissue-specific modulation of SIRT1 and a protective effect of the buckwheat diet against BPA-induced depletion in metabolically active tissues (liver and kidney), while revealing a divergent response in gastric tissue.

Molecular docking results

Molecular docking simulations using CB-Dock2 revealed favorable binding interactions of rutin (SANC01085) and quercetin (SANC00317) with the SIRT1 (4I5I. pdb) protein structure. Rutin displayed the strongest binding energy of –10.4 kcal/mol (best pose in cavity 2), while quercetin exhibited –9.5 kcal/mol (best pose in cavity 1).

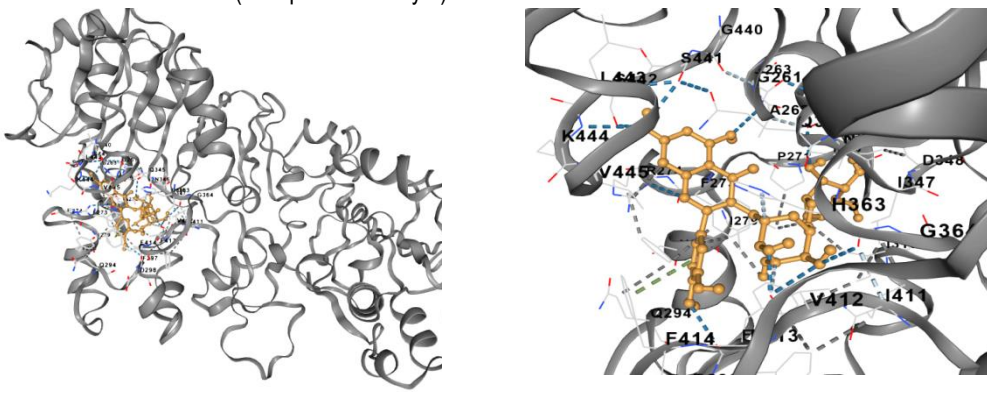


Figure 3. 3D visualization of the molecular docking interaction between rutin and the SIRT1 protein

Both compounds shared a common set of interacting residues in the catalytic proximal and allosteric region near the NAD^+-binding site, including GLY261, ALA262, SER265, CYS268, PHE273, ARG274, TYR280, GLU315, PHE321, SER324, LEU325, GLN345, ASN346, ILE347, ASP348, HIS363, VAL412, PHE413, PHE414, GLY440, SER441, SER442, ASP481, and ASP483.

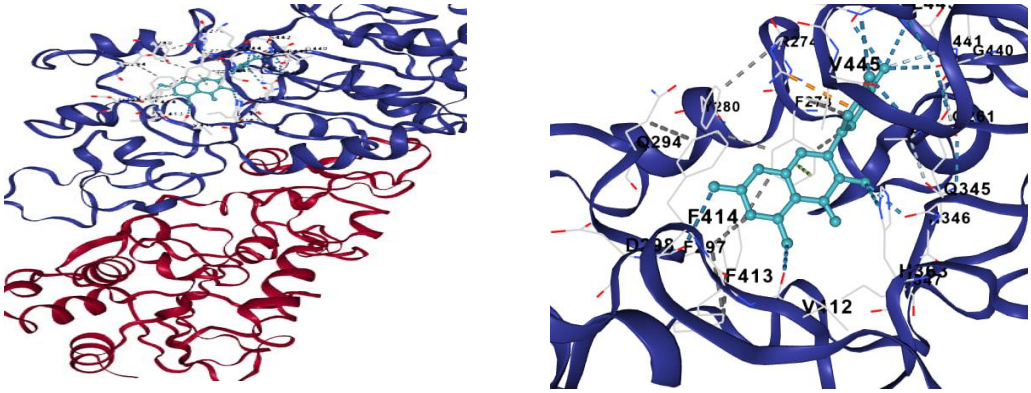


Figure 4. 3D visualization of the molecular docking interaction between quercetin and the SIRT1 protein

The marginally stronger binding affinity of rutin is consistent with its substantially higher concentration in the buckwheat extract (1.032 mg/g versus 0.308 mg/g for quercetin) and provides a plausible molecular mechanism underlying the observed in vivo modulation of SIRT1 protein levels.

4. Discussion

The present study demonstrates that an exclusive buckwheat diet (*Fagopyrum esculentum*; 50:50 mixture of whole and ground seeds) exerts tissue-specific modulation of SIRT1 protein levels in male albino mice, both under basal conditions and during exposure to bisphenol A (BPA; 15 mg/L in drinking water for 25 days). The findings reveal a partial protective effect against BPA-induced SIRT1 depletion in metabolically active tissues (liver and kidney), while showing a distinct normalization in gastric tissue.

Phytochemical composition and biological relevance

HPLC quantification identified rutin as the dominant flavonoid in 70% ethanolic buckwheat extracts (1.032 ± 0.221 mg/g), followed by dihydroquercetin (0.404 ± 0.102 mg/g) and quercetin (0.308 ± 0.086 mg/g). LC-MS analysis further confirmed the presence of kaempferol-3-O-rutinoside (m/z 525.18), isoorientin/vitexin, chlorogenic acid, caffeic acid, and quinic acid. These results align closely with recent phytochemical profiling of common buckwheat [6, 14, 23], which consistently highlight rutin and quercetin as major contributors to its antioxidant and anti-inflammatory capacity. Rutin and quercetin are well-established direct activators of SIRT1, promoting NAD^+-dependent deacetylation and downstream effects on PGC-1α-mediated mitochondrial biogenesis [8, 10]. The high abundance of these compounds in the administered diet likely underpins the observed biological responses.

Effects in liver and kidney tissues

Under normal conditions, the buckwheat diet modestly reduced SIRT1 levels in the liver (3.94 ± 0.08 vs. 4.342 ± 0.21 ng/mL) and kidney (4.73 ± 0.16 vs. 5.40 ± 0.04 ng/mL). BPA exposure alone significantly lowered SIRT1 content, consistent with prior reports of BPA-induced NAD^+ depletion and activation of pro-inflammatory pathways (TNF-α, NF-κB) in these organs [21]. Co-administration of buckwheat with BPA not only reversed this decline but elevated SIRT1 above control levels (liver: 4.67 ± 0.19 ng/mL, +25.2%; kidney: 5.91 ± 0.10 ng/mL, +9.4%). These restorative effects corroborate earlier findings that buckwheat-enriched diets attenuate BPA-induced oxidative stress and upregulate SIRT1 and antioxidant enzymes [15, 19]. The protective action is most likely mediated by the direct SIRT1-activating properties of rutin and quercetin, in combination with their broad antioxidant and anti-inflammatory activities.

Distinct response in stomach tissue

In contrast, BPA exposure increased SIRT1 levels in stomach tissue (+7.1% vs. control), whereas buckwheat-containing diets (alone or with BPA) normalized values toward or slightly below control levels. This divergent pattern may reflect tissue-specific mechanisms: BPA is known to activate estrogen receptors (ERα/ERβ) in gastric mucosa, potentially triggering transient local inflammation and compensatory upregulation of sirtuins [16, 19, 26]. Differential absorption, first-pass metabolism, or local biotransformation of flavonoids in the stomach may also contribute to the attenuated response compared to liver and kidney. Similar tissue-specific modulation of the PGC-1α/SIRT1 axis has been reported with tartary buckwheat in metabolic stress models [11].

Significance of molecular docking findings

In silico docking using CB-Dock2 confirmed strong binding affinities of rutin (–10.4 kcal/mol) and quercetin (–9.5 kcal/mol) to the catalytic proximal and allosteric regions of SIRT1, near the NAD^+-binding site. Both compounds interacted with a shared set of key residues (GLY261, ALA262, SER265, CYS268, PHE273, ARG274, TYR280, GLU315, PHE321, SER324, LEU325, GLN345, ASN346, ILE347, ASP348, HIS363, VAL412, PHE413, PHE414, GLY440, SER441, SER442, ASP481, ASP483). These results are in agreement with recent docking and dynamics studies [1, 10, 17] and provide a molecular basis for the direct activation potential of buckwheat flavonoids, with rutin's superior affinity correlating with its higher abundance in the extract.

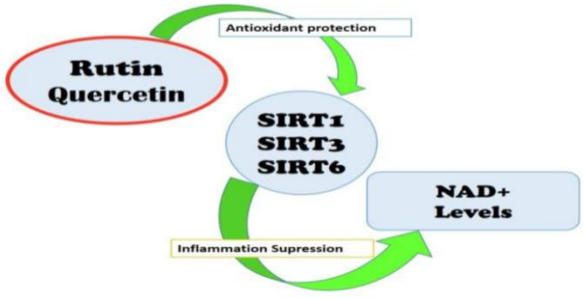


Figure 5. Cyclic mechanism of rutin and quercetin on SIRT1/SIRT3/SIRT6 activation, NAD^+ levels, antioxidant protection, and

inflammation suppression. Rutin and quercetin from buckwheat activate SIRT1, SIRT3, and SIRT6, elevating NAD^+ levels. This enhances antioxidant defenses (reduced ROS, increased SOD/CAT/GPx) and suppresses inflammation (downregulated NF-κB/TNF-α pathways), forming a positive feedback loop. The model integrates our findings of tissue-specific SIRT1 restoration under BPA exposure with flavonoid-mediated direct activation and broader protective effects.

Caloric and compositional considerations

Buckwheat differs from standard rodent chow in fiber content (20–30%), lower protein and fat, and reduced caloric density (350 kcal/100 g vs.

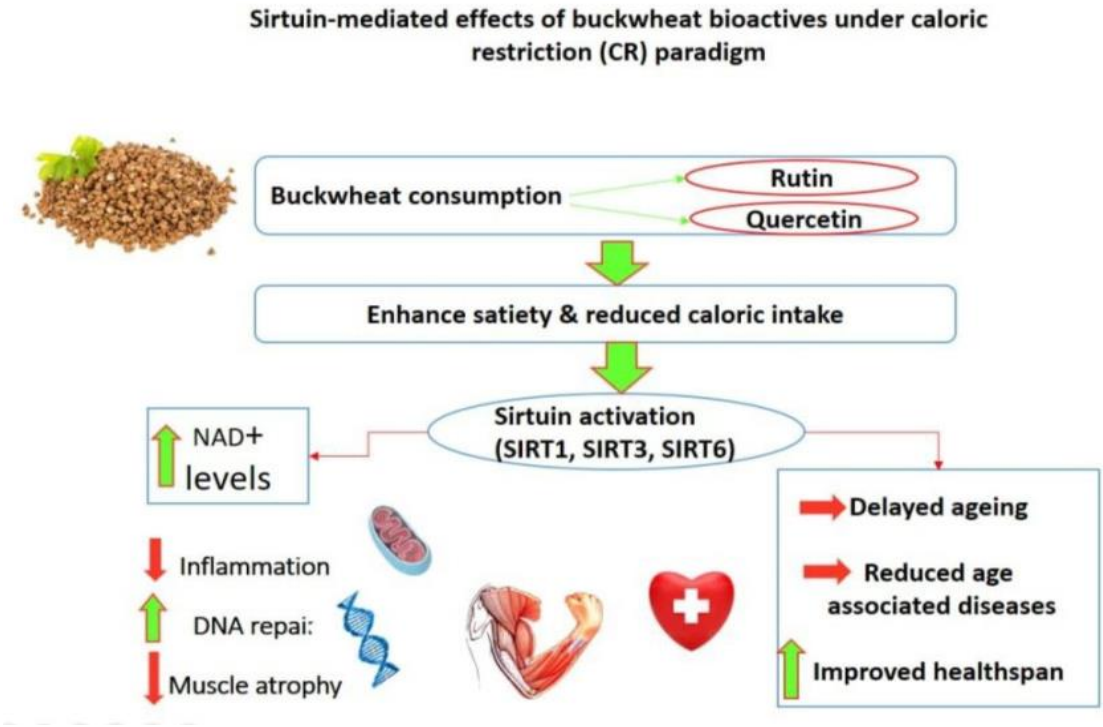


Figure 6. Sirtuin-mediated effects of buckwheat bioactives under a caloric restriction (CR)-mimetic paradigm.

400–450 kcal/100 g) [24]. Such differences may mimic caloric restriction-like effects, including elevated NAD^+ and SIRT1 activation [3]. However, food intake and energy expenditure were not quantified, limiting attribution of effects solely to flavonoids. Future studies should incorporate pair-feeding or indirect calorimetry to distinguish these mechanisms. Buckwheat consumption, rich in rutin and quercetin, promotes satiety and may reduce caloric intake naturally. This leads to elevated NAD^+ levels, activating sirtuins (SIRT1, SIRT3, SIRT6), which in turn reduce inflammation, enhance DNA repair, prevent muscle atrophy, and promote mitochondrial biogenesis. These mechanisms contribute to delayed aging, reduced age-associated diseases, and improved healthspan. The diagram aligns with our findings of tissue-specific SIRT1 restoration/elevation in liver and kidney under BPA exposure, likely mediated by direct sirtuin-activating properties of buckwheat flavonoids combined with CR-like effects.

Regulatory and Public Health Implications of BPA Exposure

The findings of the present study should also be considered in the context of food safety regulation. Bisphenol A (BPA) is used in the manufacture of certain food-contact materials, including epoxy resin coatings, plastics, printing inks, adhesives, and other materials. BPA may migrate from these materials into food, creating a potential route of human exposure [30]. In response to the safety concerns associated with BPA, the European Commission adopted Regulation (EU) 2024/3190, which establishes restrictions on the use of BPA and certain other hazardous bisphenols in specified materials and articles intended to come into contact with food. The regulation entered into force on 20 January 2025 and includes transitional provisions for certain products [30].

In Uzbekistan, the Law No. 483-I of 30 August 1997, *On the Quality and Safety of Food Products*, provides a general legal

framework for food safety. Its provisions include state regulation, registration of food products and equipment intended for food production and contact with food, as well as state supervision and control. The law also encompasses packaging and auxiliary materials that come into contact with food products [31]. These provisions provide a general legal basis for controlling food safety; however, the existence of this framework should not be interpreted as evidence of a specific national prohibition of BPA equivalent to the EU measure.

The tissue-specific changes in SIRT1 protein concentrations observed in the present mouse model provide experimental evidence of biological responses under the applied BPA exposure conditions. Nevertheless, these findings do not establish the level of human risk associated with BPA exposure from food-contact materials, nor do they determine a safe or harmful exposure threshold. Further studies involving exposure assessment, chemical migration analysis, and validated dose–response investigations are required to support evidence-based risk assessment and regulatory decision-making. Thus, the present findings may contribute to the broader scientific discussion on BPA toxicity and food safety, while their regulatory implications should be interpreted cautiously.

Limitations and future directions Key limitations include (1) lack of caloric intake and NAD^+ measurements, (2) use of only male animals (precluding sex-specific analysis), (3) relatively short exposure duration (25 days), and (4) absence of additional inflammatory (IL-6, NF-κB) or estrogen receptor markers to explain gastric responses. Future investigations should assess NAD^+/NADH ratios, reactive oxygen species, long-term chronic exposure, and sex differences to strengthen mechanistic understanding.

5. Conclusion

This study provides evidence of tissue-specific modulation of SIRT1 protein levels by an exclusive buckwheat diet in mice, with clear protective restoration against BPA-induced depletion in liver and kidney and normalization in stomach tissue. Phytochemical profiling confirmed high levels of bioactive flavonoids (particularly rutin and quercetin), while molecular docking supported their direct interaction with SIRT1. These findings highlight the potential of flavonoid-rich whole-food interventions, such as buckwheat, in mitigating environmental toxicant stress and suggest promising applications in nutritional strategies for protection against endocrine-disrupting chemicals.

Conflict of Interest

The authors declare no conflicts of interest.

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