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Germinated Fava Bean (*Vicia faba* L.) extract attenuates acrylamide-induced neuroinflammation in rats via antioxidant, anti-inflammatory, and neurotransmitter modulation

Hend A. Essa^{1*}, Walaa M. El Sayed² and Ahmed Fotouh³

Abstract

Acrylamide (ACR) is a neurotoxic and carcinogenic compound found in many foods and industrial products, raising significant public health concerns. It induces oxidative stress, inflammation, and neurotransmitter disruption, all of which contribute to neuroinflammation. This study assessed the neuroprotective potential of germinated faba beans (*Vicia faba* L., cultivar Giza 843) against ACR-induced neurotoxicity in a rat model and compared two methods of germination (traditional germination (in light) and (germination in the dark)) to enhance their neuroprotective properties. The total phenolic, flavonoid, and antioxidant contents, as well as the profile of faba beans, were assessed via high-performance liquid chromatography (HPLC) and an amino acid analyser. Thirty-two Sprague–Dawley rats ($n=8/\text{group}$) were randomly divided into the following groups: control, ACR-exposed, ACR coadministered with traditionally germinated fava bean extract (GFB), and ACR coadministered with ungerminated fava bean extract. Neuroinflammation was induced via the oral administration of ACR (35 mg/kg/day for 12 days), while GFB and ungerminated extracts were coadministered at 300 mg/kg/day for 12 days. Gait scores were assessed. Antioxidants (GSH, SOD), oxidative stress markers (MDA, NO), inflammatory cytokines (TNF- α , IL-6), neurotransmitters (GABA, serotonin, dopamine), and acetylcholinesterase activity were measured in brain tissues (cerebrum, cerebellum, hippocampus). Histopathology and immunohistochemistry (with anti-GFAP and anti-caspase-3 antibodies) were performed to assess structural damage and apoptotic cell death. The results revealed that, traditionally, germination yielded relatively high levels of bioactive compounds and increased antioxidant activity. Compared with the ACR and ungerminated groups, the optimised traditional germinated faba bean extract significantly reduced oxidative stress, inflammation, and neurotransmitter disruption. The increased neuroprotection was attributed to increased polyphenolic, flavonoid, and amino acid levels from traditional germination. The neuroprotective potential of faba beans against ACR toxicity can be enhanced through traditional germination methods. This finding presents a compelling, natural therapy for mitigating the serious public health concerns associated with ACR exposure, and further clinical trials are therefore needed.

Keywords Faba beans, Neuroinflammation, Germination, Neurotransmitter, Acrylamide, Oxidative stress

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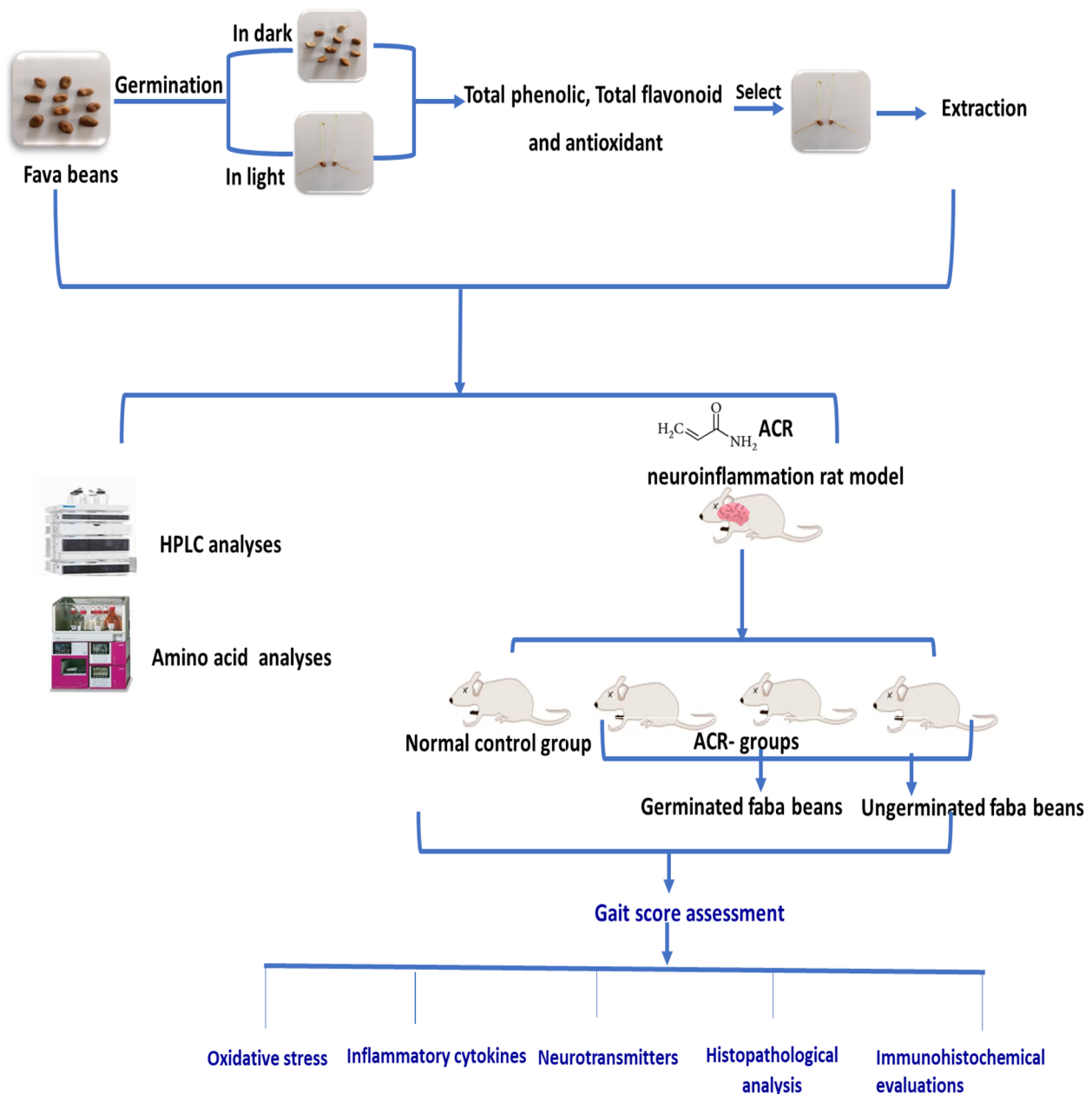
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Graphical Abstract**Introduction**

Acrylamide (ACR) is a well-documented neurotoxic and carcinogenic compound. Owing to its widespread presence in dietary and industrial settings, it poses significant public health risks. ACR forms primarily during the high-temperature cooking of starchy foods through the Maillard reaction and are commonly found in baked

and fried carbohydrate-rich foods such as bread, breakfast cereals, potato chips, and coffee (Koszucka et al., 2020; Ghasemzadeh Rahbardar et al., 2021). The average daily intake of ACR for adults is approximately 0.5 µg/kg body weight, which amounts to 14–70 µg/day for a 70 kg individual. Alarming, children consume even higher amounts, ranging from 70 to 280 µg/day (Ghasemzadeh

Rahbardar et al., 2021). This widespread dietary exposure is concerning, as ACR has been linked to cytotoxicity (Kianfar et al., 2020), neuroinflammation (Yazadanpanah et al., 2023), and carcinogenicity (Koszucka et al., 2020).

In addition to dietary sources, ACR is a synthetic vinyl monomer widely used in industrial applications, including wastewater treatment, ore processing, and cosmetic production (Dybing et al., 2005). Subchronic exposure to ACR can lead to severe neuropathies characterised by ataxia, numbness in the extremities, muscle weakness, and gait irregularities (Pennisi et al., 2013). It also causes neuronal loss in both the central and peripheral nervous systems (Wei et al., 2015). Occupational exposure studies have reported neurotoxic symptoms such as sensory disturbances, locomotor defects, and weight loss in workers (Matoso et al., 2019). Moreover, a recent cohort study involving 2534 elderly individuals suggested that ACR intake may contribute to cognitive decline in older Chinese men (Liu et al., 2017), underscoring the global concern over the neurotoxic effects of ACR.

The primary mechanism underlying ACR-induced neuroinflammation is oxidative stress (Erkekoglu & Baydar, 2014). ACR disrupts cellular redox homeostasis by increasing reactive oxygen species and nitric oxide synthase levels, leading to lipid peroxidation and the accumulation of malondialdehyde and nitric oxide. Concurrently, it depletes glutathione and inhibits antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Erkekoglu & Baydar, 2014). This oxidative damage extends to intracellular proteins and lipids, triggering neuronal death and synaptic failure (Kopanska et al., 2018). Additionally, ACR-induced oxidative stress activates microglia and astrocytes, resulting in the release of proinflammatory cytokines such as tumor necrosis factor- α and interleukin-6, which exacerbate neuronal damage (Zong et al., 2019). ACR also disrupts neurotransmitter levels, impairing synaptic transmission in the central nervous system (Agrawal et al., 1981).

Given the severity of ACR-induced neuroinflammation, there is an urgent need to identify natural interventions that can mitigate its harmful effects. Faba beans (*Vicia faba* L.), one of the world's oldest cultivated crops, have been a dietary staple for thousands of years, with evidence of their use in ancient Egypt, China, and Mediterranean cultures (Amarowicz et al., 1996). These beans are rich in protein, fibre, carbohydrates, minerals, and secondary metabolites such as phenolics, which contribute to their high nutritional value (Amarowicz et al., 1996). Notably, faba beans are a source of essential amino acids, including aspartic acid, threonine, serine, methionine, and tyrosine, which play critical roles in neurotransmitter synthesis and neuronal function (Etemadi et al., 2018). Germination further enhances the bioactive compounds

in faba beans, including phenolics, flavonoids, and amino acids, significantly increasing their antioxidant potential (Mehran & Golshani, 2013; Randhir & Shetty, 2004). This makes germinated faba beans promising candidates for combating oxidative stress and inflammation, two key drivers of ACR-induced neuroinflammation.

This study evaluated germinated faba beans on the basis of evidence from traditional practices and scientific research. In Mediterranean and Middle Eastern cultures, faba beans have long been recognised for their nutritional and medicinal benefits. Scientific investigations indicate that germination increases the levels of bioactive compounds such as phenolics, flavonoids, and amino acids, which contribute to reducing oxidative stress and inflammation (Goyal et al., 2020; Randhir & Shetty, 2004). Furthermore, recent research has demonstrated that plant-derived antioxidants can reduce neuroinflammation and improve cognitive function in animal models (Essa et al., 2024; Kumar et al., 2022; Zhang et al., 2021). These findings collectively support the potential of germinated faba beans as a natural intervention for ACR-induced neuroinflammation.

This study explored the neuroprotective effects of germinated faba beans from the Egyptian cultivar Giza 843 against ACR-induced neuroinflammation in a rat model, with a focus on oxidative stress, inflammation, and neurotransmitter modulation. This study bridges a critical gap in the literature by providing a scientific basis for the use of germinated faba beans as a natural dietary intervention against ACR-related neuroinflammation, with significant public health implications for mitigating ACR-associated risks.

An initial *in vitro* study compared two germination methods, traditional germination (in light) and germination in the dark, to enhance their neuroprotective properties. On the basis of chemical analysis, the germination method, which yields relatively high levels of phenolics, flavonoids, antioxidants, and amino acids, was selected for subsequent *in vivo* studies using a rat model of ACR-induced brain toxicity. This research bridges traditional knowledge of the medicinal properties of faba beans with modern evidence of their neuroprotective potential, offering a model for leveraging plant-based interventions to counteract environmental toxins.

Materials and methods

Materials

Plant materials

Faba bean seeds (Giza 843 cultivar) were obtained and identified by Prof. Dr. Gamal Abdel Aziz (Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt). Prof. Dr. Mona Mohamed Marzouk deposited

voucher specimens (M274) in the National Research Centre (CAIRC) herbarium.

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) and acrylamide were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All other chemical reagents and solvents were of high analytical grade.

Methods

In vitro assay

Germination of Faba bean

Faba bean seeds were cleaned, sorted, washed, and soaked for 24 h, and the soaking water was changed every 2 h for 3 consecutive times. The water was separated well, and the seeds were placed in a muslin cloth for germination. Faba bean seeds were germinated at room temperature (20–25 °C) for 10 days. The germination process is carried out in two ways. The first method involves germination in the dark by covering seeds with a muslin cloth and keeping them in a dark place (Kassegn et al., 2018). The second method of germination is traditional germination (Hamza et al., 1987), where the seeds are left in the light during the day (Fig. 1). During germination, the seeds were sprayed with water twice daily. After germination, the Faba was dried in an oven at 50 °C until drying. The dried Faba beans were milled, sieved and packed into polyethylene bags and stored at –18 °C until use.

Extraction

Both the raw and germinated seeds (500 g each) were ground into a fine powder. This powder was then subjected

to sonication-assisted extraction via 80% ethanol (3 × 3 L for 30 min) and subsequently filtered. The combined filtrate was centrifuged, and the supernatant was concentrated via a vacuum rotary evaporator (Mekky et al., 2020).

Determination of total phenolic and total flavonoid contents

The total phenolic compounds were determined via the method described by Kaur and Kapoor (2002). Two hundred microliters (200 µL) of a 70% methanolic extract (1 mg/mL) was brought to a final volume of 3 mL with distilled water. This mixture was then thoroughly mixed with 0.5 mL of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 mL of a 20% (w/v) sodium carbonate solution. The resulting mixture was allowed to stand in the dark for an additional 60 min, after which the absorbance was measured at 650 nm. The test was performed in triplicate for each sample. The total phenolic content was calculated via a calibration curve, and the results were expressed as milligrams of gallic acid equivalent per gram of dry weight.

The total flavonoid content was determined via the method described by Karawya and Aboutabl (1982). One gram (1 g) of defatted dried powder from each sample was placed in a beaker, and 95% ethyl alcohol was added. The solution was then filtered, and the filtrate was concentrated in a water bath until the volume was reduced to less than 50 mL (10–20 mL). The volume was then adjusted to 50 mL with 95% ethyl alcohol to create a stock solution. From this stock solution, 0.5 mL was transferred to a test tube and evaporated to complete dryness in a water bath. Subsequently, 5 mL of 0.1 M aluminum chloride (ALCL₃) was added, and the mixture was mixed

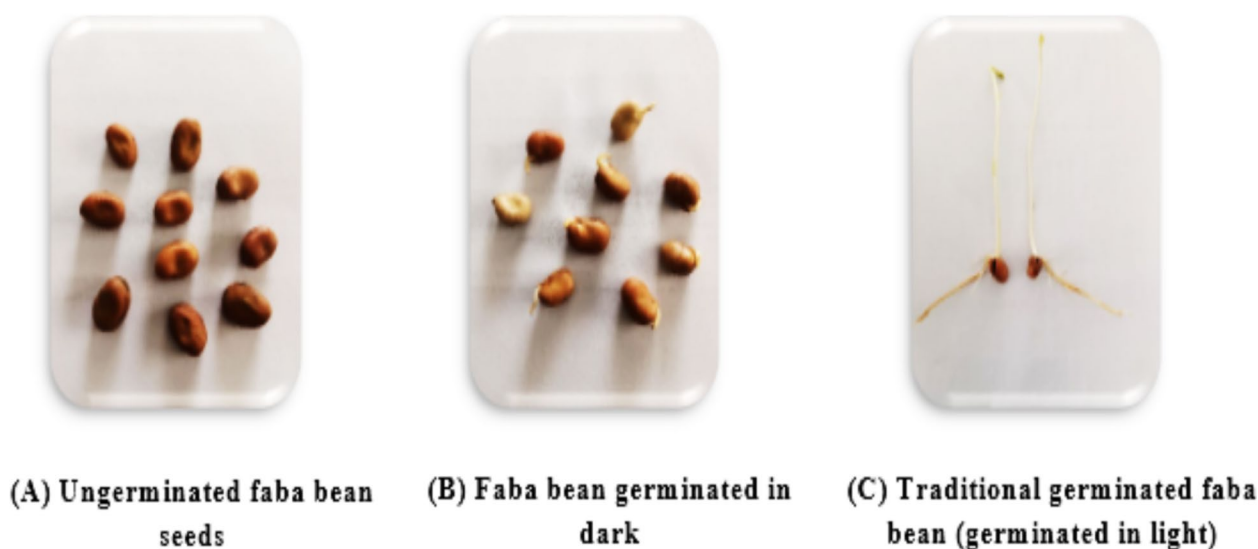


Fig. 1 Photograph of (A) ungerminated faba bean seeds, B Faba bean germinated in the dark at the end of germination and (C) traditional germinated faba bean at the end of germination

well. The test was performed in triplicate for each sample. Readings were taken at two wavelengths:

$\lambda = 445$ nm (quercetin) range of the calibration curve (0.19–0.97).

$\lambda = 226$ nm (kaempferol) range of the calibration curve (0.033–0.185).

The total flavonoid content was calculated according to the following equation:

$$X = y + 0.0195/0.0029.$$

X = concentration (ppm), Y = absorbance.

In vitro antioxidant activity 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging activity of the extracts was assessed via the DPPH assay, which measures the capacity to donate hydrogen atoms or electrons on the basis of the decolorisation of a purple methanol–DPPH solution. Spectrophotometric analysis was performed according to the method described by Burits and Bucar (2000). Briefly, 50 μ L of the extracts (at various concentrations) were added to 5 mL of the DPPH solution (0.004% in methanol). After a 30-min incubation period at room temperature, the absorbance was read against pure methanol at 517 nm. The test was performed in triplicate for each sample. The radical-scavenging activities of the samples were calculated as the percentage of inhibition via the following equation:

$$I\% = (A \text{ blank} - A \text{ sample})/A \text{ blank} \times 100$$

where I% is the radical-scavenging activity of the samples and A blank is the absorbance of the control (containing all reagents except the test compound).

High-performance liquid chromatography (HPLC) analysis of phenolic and flavonoid compounds

A Thermo Ultimate 3000 HPLC system equipped with a pump, autosampler, DAD-3000 diode array detector, and Chromelion 7 software was used. Separation was carried out on a Thermo-Hypersil C18 column (2.5 $\times$ 30 cm, 25 $^{\circ}$ C) with a mobile phase of 0.05% trifluoroacetic acid/acetonitrile (solvent A) and distilled water (solvent B). Detection was performed at 230–400 nm. The samples, standards, and mobile phase were degassed and filtered (0.45 μ m, Millipore). The compounds were identified by comparing their retention times and UV spectra with those of reference standards (Biswas et al., 2013). The test was performed in triplicate for each sample.

Determination of the amino acid profile

Amino acid analysis was conducted following the methods of Csomos and Simon-Sarkadi (2002). Briefly, defatted and dried samples (0.2 g) were hydrolysed with 6 N HCl (10 mL) at 105 $^{\circ}$ C for 12 h in sealed tubes. The hydrolysate was diluted to 25 mL with deionised water, filtered, and evaporated to remove HCl. The samples and standards were reconstituted in citrate buffer (pH 2.2) and analysed via an INGOS amino acid analyser. The test was performed in triplicate for each sample.

In vivo assay

Neuroinflammation animal model

Ethics statement

The study was approved by the General Organisation for Teaching Hospitals and Institutes (GOTHI) Ethics Committee (Approval No. IN000147) and complied with the National Institutes of Health guidelines for the care and use of laboratory animals (Garber et al., 2011).

Animals and diet

Female Sprague–Dawley rats (4–6 weeks old, 220–250 g) were obtained from the animal facility of the Ophthalmology Research Institute, Egypt. Following a one-week acclimatisation period, the rats were housed individually in stainless steel cages under standard laboratory conditions: temperature 22 ± 2 $^{\circ}$ C, relative humidity 55–60%, and a 12-h light/dark cycle. The animals were fed a standard laboratory diet and provided tap water ad libitum.

The rats were fed a well-balanced diet formulated according to Reeves et al. (1993), consisting of 12% casein-derived protein, 10% corn oil, 10% sucrose, 58.5% corn starch, 5% cellulose, 3.5% AIN-93 salt mixture, and 1% AIN-93 vitamin mixture.

Animal experiments

After one week of acclimatisation, 32 rats were randomly divided into four groups ($n = 8/\text{group}$) as follows:

- Group 1 (normal control) received saline (0.5 mL/day, P.O.) and a standard diet.
- In Group 2 (the ACR group), acrylamide (35 mg/kg/day, P.O. dissolved in 0.5 mL of saline) was administered for 12 days at 9:00 a.m. (Fang et al., 2022).
- Group 3 (germinated faba bean): Co-administered acrylamide (35 mg/kg/day, P.O. dissolved in 0.5 mL saline at 9:00 a.m.) and germinated faba bean extract (300 mg/kg/day, P.O. at 2:00 p.m.) for 12 days (Abdel-Sattar et al., 2021).
- Group 4 (ungerminated faba bean): Acrylamide (35 mg/kg/day, P.O. dissolved in 0.5 mL saline at 9:00 a.m.) and ungerminated faba bean extract (300 mg/

kg/day, P.O. at 2:00 p.m.) were coadministered for 12 days (Abdel-Sattar et al., 2021).

The rats were given access to feed and tap water ad libitum. By the end of the experiment, total food intake, body weight gain, the feed efficiency ratio, and relative brain weight were determined (Chapman et al., 1959).

Assessment of gait scores

At the end of the experiment, gait abnormalities were evaluated via the scoring system of LoPachin et al. (2002). The rats were observed for 3 min on a flat surface, and gait was scored as follows:

- Score 1: Normal gait.
- Score 2: Mild hindlimb weakness, slight ataxia, and foot splay.
- Score 3: Moderate gait impairment with reduced activity and foot splay.
- Score 4: Severe impairment, including hindlimb dragging and pronounced weakness.

Brain sampling and homogenate preparation

Following gait assessment, the animals were anaesthetised via intraperitoneal (i.p.) injection of sodium pentobarbital at a dosage of 50 mg/kg. Euthanasia was performed via decapitation. Whole brains were excised, washed in ice-cold saline, dried with filter paper, weighed, and immediately placed onto a chilled plate for dissection into three distinct regions: the cerebrum, cerebellum, and hippocampus. Each region was then divided into two portions for subsequent analyses.

The first portion was homogenised (10% w/v) in ice-cold phosphate buffer (pH 7.4) via a tissue homogeniser (MPW-120, BitLab Medical Instruments, Poland). The homogenates were centrifuged (4000 rpm, 10 min, 4 °C) in a cooling centrifuge (Laboratory Centrifuge, 2K15, Sigma Co., Germany) (Essa et al., 2025a, b). The supernatants were aliquoted and stored at −20 °C for biochemical analyses, including analysis of oxidative/antioxidant markers, neurotransmitter levels, and inflammatory cytokines. The second portion was fixed in 10% neutral buffer formalin for histopathological and immunohistochemical examination.

Evaluation of oxidative/antioxidant parameters

The oxidative status of brain tissue homogenates was assessed by measuring the levels of malondialdehyde (MDA), a marker of lipid peroxidation, superoxide dismutase (SOD), reduced glutathione (GSH), and nitric oxide (NO). These parameters were evaluated

spectrophotometrically via the methods of (Nair & Turner, 1984; Sun et al., 1988; Jollow et al., 1974; Montgomery & Dymock, 1961). The optical density of all the measured parameters was determined via a spectrophotometer (Shimadzu UV-2401 PC, Australia). Spectrophotometric analyses were conducted in accordance with the instructions provided in the assay kits.

Evaluation of TNF- α and IL-6 in brain tissue

The levels of TNF- α (Cat. No. SL0722Ra) and IL-6 (Cat. No. SL0411Ra) were measured via rat-specific enzyme-linked immunosorbent assay (ELISA) kits (Sunlong Biotechnology Co., Ltd., Hangzhou, China) following the manufacturer's protocols.

Determination of dopamine, serotonin, acetylcholinesterase and gamma-aminobutyric acid in brain homogenates

Dopamine (Cat. No. SL0243Ra) and serotonin (Cat. No. SL1046Ra) levels, along with acetylcholinesterase (AChE) activity and gamma-aminobutyric acid (GABA) content, were quantified in brain homogenates via rat-specific ELISA kits (Sunlong Biotechnology Co., Ltd., Hangzhou, China) following the manufacturer's protocols (AChE: Cat. No. SL0027Ra; GABA: Cat. No. SL0299Ra).

Microscopic examination

First, proper fixation of cerebral, cerebellar, and hippocampal tissue samples was performed. The handling of fresh brain tissue should be minimised before fixation to avoid artifacts such as “dark neurons,” which result from mechanical trauma. Proper orientation of the hippocampus was critical to ensuring consistent sectioning across samples and valid comparisons between groups. The hippocampus has a 3D curved structure, and the sections must be taken in a standardised coronal (or horizontal/sagittal) plane. The samples were dehydrated in a series of ascending grades of alcohol, cleared in xylene, and then embedded in paraffin. Paraffin blocks were prepared, and 5 μ m thick tissue sections were obtained, which were then deparaffinized and stained with hematoxylin and eosin. The obtained sections were viewed, and photomicrographs were captured and collected using a Leica DM500 microscope equipped with a digital camera according to the method described by Shosha et al. (2024).

Immunohistochemical staining of GFAP and caspase-3

The avidin–biotin complex (ABC) technique was used for immunohistochemical detection of GFAP and caspase-3 within the rat brain. Dewaxed and dehydrated sections were incubated overnight at 4 °C with rabbit anti-GFAP and anti-caspase-3 antibodies (sc-33673; Santa Cruz

Table 1 Total phenolics, total flavonoids and DPPH in extracts of ungerminated, dark and traditional germinated faba bean seeds at the end of germination

Components (%)	Faba bean		
	Ungerminated	Germinated in dark	Traditional germinated
Total phenolics	0.74 ± 0.01 ^c	1.25 ± 0.02 ^b	2.77 ± 0.02 ^a
Total flavonoids	0.17 ± 0.02 ^c	0.20 ± 0.01 ^b	0.29 ± 0.01 ^a
DPPH	43.08 ± 0.02 ^c	66.36 ± 0.05 ^b	86.98 ± 0.03 ^a

The data are presented as the means ± standard deviations (*n* = 3). In each row, identical letters indicate statistically insignificant differences, whereas different letters indicate statistically significant differences (*p* ≤ 0.05) between ungerminated faba bean seeds and dark-germinated and traditional germinated seeds according to Duncan's post hoc test

Biotech, CA, USA) at a ratio of 1:200. Visualisation was performed via commercial ABC (Santa Cruz Biotech, CA, USA) for 30 min. Next, the sections were subjected to 3,3'-diaminobenzidine tetrahydrochloride (DAB) as the chromogen and then counterstained with haematoxylin. Positive GFAP and caspase-3 reactions were detected as brown colouration. According to Fotouh et al. (2024),

Qualitative evaluation of GFAP and caspase-3 immunostaining

Qualitative scores of GFAP and caspase-3 immunostaining intensity were negative or not (-), minimal (+), moderate (++) and strong (+++) as outlined by Smolen (1990). The intensity of the brown color of DAB was expressed as the relative optical density of the diaminobenzidine reaction product.

Statistical analysis

Statistical analyses were conducted via SPSS version 25. For the in vivo data (*n* = 8 per group), normality assumptions were checked via the Shapiro–Wilk test before analysis. The parametric data are presented as the means ± standard errors (SEs) and were analysed via one-way analysis of variance (ANOVA), followed by Duncan's post hoc test to identify specific differences between groups. A significance level of *P* ≤ 0.05 was used to assess statistical significance. The Kruskal–Wallis H test was used for nonparametric comparisons of frequency data, followed by Dunn's post hoc test to determine specific group differences. The results for nonparametric data are reported as median values.

Results

In vitro

The contents of total phenolics, total flavonoids, and DPPH radical scavenging activity in the faba bean seeds and differently germinated samples are presented in Table 1. Compared with those of ungerminated seeds, significant differences (*P* ≤ 0.05) in antioxidant activity were detected among germination methods, with

traditionally germinated faba beans showing the highest values for all measured parameters.

Table 1 shows that traditionally germinated faba beans had a higher total phenolic content (2.77%) than dark-germinated (1.25%) and ungerminated seeds did (0.74%). The total flavonoid content followed the same pattern: traditional germination (0.29%), dark germination (0.20%), and ungerminated seeds (0.17%). This trend held for antioxidant activity according to the DPPH assay: traditionally germinated beans had the highest radical scavenging capacity (86.98%), followed by dark-germinated (66.36%) and ungerminated seeds (43.08%).

HPLC analysis (Table 2) revealed significant differences (*P* ≤ 0.05) in phenolic and flavonoid compositions between ungerminated and germinated faba beans. Germination markedly increased the contents of most compounds, with traditional germination yielding the highest levels of key flavonoids: diosmin (1.33 mg/g) and hesperidin (1.07 mg/g) were uniquely detected in traditionally germinated seeds, whereas rutin and apigenin progressively increased from dark to traditional germination. The phenolic acid content followed a similar trend, with cinnamic acid content increasing > 20-fold after germination. Notably, chlorogenic acid and flavonols (kaempferol) appeared exclusively in traditional germination, suggesting light-dependent biosynthesis activation (Fig. 2).

The germination process modifies the amino acid composition of faba beans, serving as an effective bio-fortification strategy to improve their nutritional value. Comparative analysis revealed significant differences (*P* ≤ 0.05), indicating that germination generally enhances the content of several essential amino acids, with the traditional germination method demonstrating particularly remarkable improvements Table 3.

The valine content was 53% greater than that in raw seeds (increasing to 8.49 mg/g from 5.55 mg/g), and the threonine content was 122% greater than that in ungerminated seeds (up to 4.42 mg/g from 1.99 mg/g). Most strikingly, this method produced a fivefold increase in methionine compared with that in ungerminated seeds

Table 2 HPLC profile of phenolics and flavonoids in extracts of ungerminated, dark and traditional germinated faba bean seeds at the end of germination

Components (mg/g)	Faba bean		
	Ungerminated	Germinated in dark	Traditional germinated
Phenolic compounds			
Quinic	0.0029 ± 0.00010 ^c	0.0299 ± 0.00010 ^a	0.0243 ± 0.00010 ^b
Ellagic	0.0004 ± 0.00040 ^c	0.0015 ± 0.00050 ^b	0.0019 ± 0.00050 ^a
Cinnamic	0.0003 ± 0.00050 ^c	0.0068 ± 0.00010 ^b	0.0073 ± 0.00030 ^a
chlorogenic	n.a	n.a	0.0005 ± 0.00050 ^a
flavonoids compounds			
Apeginin	0.1331 ± 0.00010 ^c	0.4136 ± 0.00050 ^a	0.3333 ± 0.00010 ^b
Rutin	0.1045 ± 0.00050 ^c	0.1466 ± 0.00010 ^b	0.1964 ± 0.00010 ^a
Diosmin	0.0790 ± 0.00050 ^c	0.1719 ± 0.00015 ^b	1.3255 ± 0.00050 ^a
Hesperidin	n.a	n.a	1.0661 ± 0.00050 ^a
Kampferol	n.a	n.a	0.8857 ± 0.00020 ^a

The data are presented as the means ± standard deviations ($n = 3$). In each row, identical letters indicate statistically insignificant differences, whereas different letters indicate statistically significant differences ($p \leq 0.05$) between ungerminated faba bean seeds and dark-germinated and traditional germinated seeds at the end of germination, according to Duncan's post hoc test

(0.39 mg/g versus 0.08 mg/g), indicating critical nutritional limitations in legumes. The traditional approach also generated the highest concentration of aspartic acid (56.18 mg/g), exceeding that of ungerminated samples by 345% and that of dark-germinated samples by 69% (Fig. 3).

Neuroinflammation animal model

Germinated and ungerminated faba bean extracts improved gait scores, nutritional parameters, brain oxidative stress, inflammation, and neurotransmitter markers in the studied groups of rats.

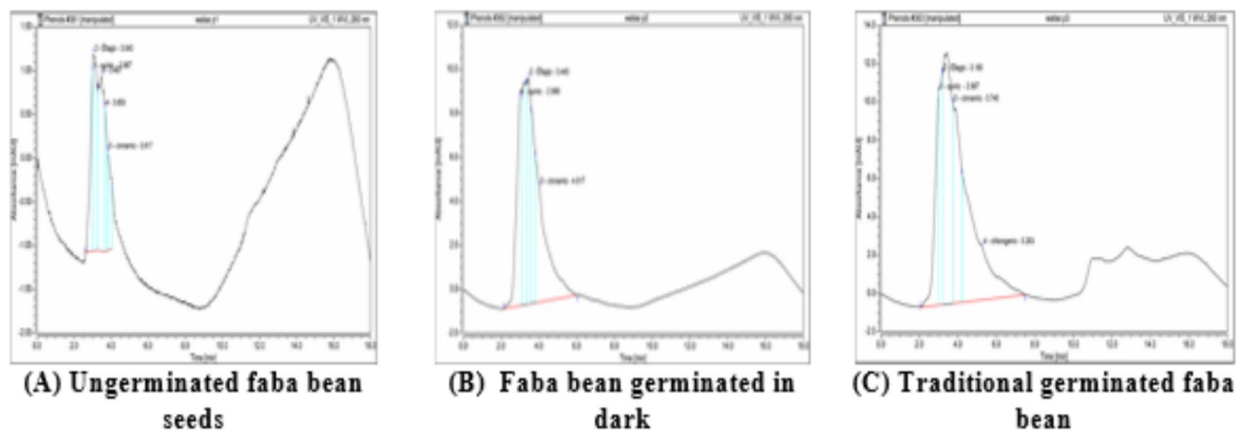
At the end of the experimental period, gait scores were assessed (Fig. 4). The rats in the normal control group exhibited normal gait patterns. In contrast, ACR-treated rats presented severe gait impairments, including foot splay, ambulation difficulties, hind limb dragging, and partial paralysis. These impairments resulted in significantly higher gait scores than those of the normal controls. However, coadministration of either germinated or ungerminated faba beans significantly improved gait abnormalities in ACR-treated rats ($p < 0.05$), reducing their gait scores to as shown in Table 4, significant ($p < 0.05$) differences in body weight, food intake, and brain weight were detected among the groups. Compared with the normal group, acrylamide-exposed rats presented a 1.26-fold increase in absolute brain weight and a 1.29-fold increase in the brain-to-body weight percentage (brain percentage), accompanied by a marked reduction in body weight gain (1.63-fold decrease) and the feed efficiency ratio (1.30-fold decrease). Compared with the acrylamide group, the treatment with germinated faba beans effectively normalised these metrics, increasing

body weight gain and the feed efficiency ratio by 1.54-fold and 1.30-fold, respectively. Ungerminated faba beans also improved these parameters, although less prominently, with a 1.27-fold increase in body weight gain and a 1.10-fold increase in the feed efficiency ratio. Both treatments significantly ($p < 0.05$) attenuated acrylamide-induced increases in brain weight and brain percentage with germinated faba beans, resulting in a 1.20-fold reduction in brain weight and a 1.24-fold decrease in brain percentage.

Table 5 presents significant ($p < 0.05$) differences in oxidative stress and inflammation in all brain regions in the acrylamide group compared with those in the normal group. In the cerebrum, acrylamide intoxication significantly induced oxidative stress and neuroinflammation. This was evidenced by an approximately 3.5-fold increase in MDA levels and an ~2.5-fold increase in NO levels compared with those in the normal group. The inflammatory response was marked by a 4.1-fold increase in IL-6 and a 3.1-fold increase in TNF- α . Concurrently, antioxidant defenses were severely compromised, with SOD activity reduced to approximately 42% of normal levels (an ~2.4-fold decrease) and GSH levels depleted to nearly 43% (an ~2.3-fold decrease). Treatment with germinated faba beans effectively mitigated this damage, reducing the elevated markers and restoring SOD and GSH to near-normal levels (e.g., MDA was reduced by approximately 65% compared with those in the acrylamide group). The ungerminated faba bean group presented an intermediate protective effect, but it was consistently less potent than the germinated treatment.

A similar pattern was observed in the cerebellum and hippocampus. Acrylamide exposure resulted in

HPLC profile of phenolics



HPLC profile of flavonoids

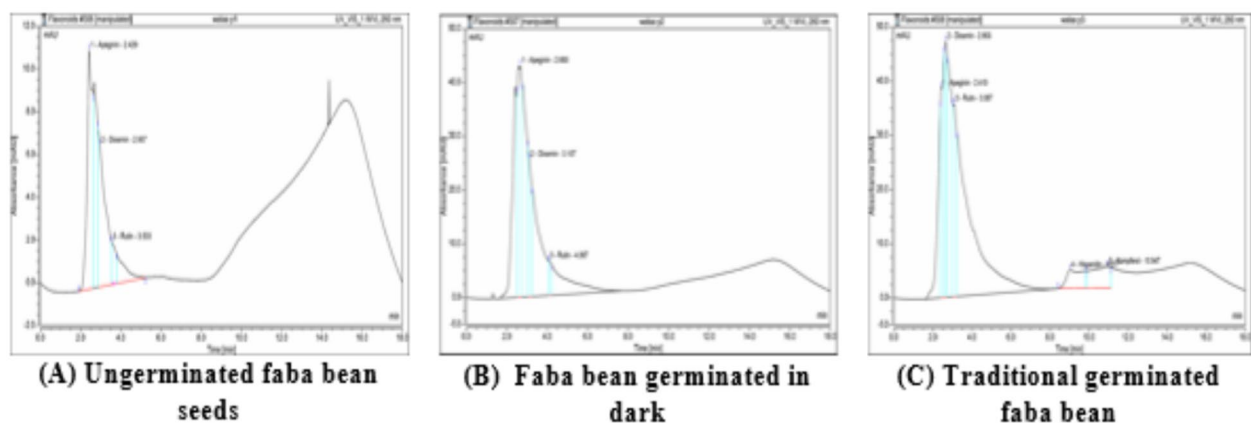


Fig. 2 HPLC profile of phenolics and flavonoids in the extracts of (A) ungerminated faba bean seeds, (B) Faba bean germinated in the dark at the end of germination and (C) traditional germinated faba bean at the end of germination

comparable fold increases; for example, MDA increased by ~3.6-fold in the cerebellum and ~3.7-fold in the hippocampus. The germinated faba bean treatment again demonstrated superior efficacy, normalising most parameters (e.g., reducing hippocampal IL-6 by over 70%) and underscoring its robust neuroprotective properties across all brain regions. The protection offered by ungerminated faba beans was partial, which is consistent with the cerebral findings.

Table 6 presents significant ($p < 0.05$) changes in neurotransmitter levels in the acrylamide group compared with those in the normal group. GABA and AChE:

The acrylamide-treated group presented pronounced increases in GABA concentrations, with levels increasing approximately 2.3-fold in all brain regions. Conversely, acetylcholinesterase (AChE) activity was markedly suppressed, decreasing to approximately 43% of normal levels (an ~2.3-fold decrease). The administration of germinated faba beans significantly ($p < 0.05$) restored GABA levels, reducing them by 1.77-fold in the cerebrum, 1.78-fold in the cerebellum, and 1.72-fold in the hippocampus, whereas AChE activity increased by 2.10-fold, 1.94-fold, and 1.95-fold, respectively, compared with that in the acrylamide group. Although ungerminated faba beans

Table 3 Amino acid profile of extracts of ungerminated, dark and traditional germinated faba bean seeds at the end of germination

Name mg/g	Faba bean			Amino acid scoring pattern (FAO, 2013)
	Ungerminated	Germinated in dark	Traditional germinated	
Essential amino acids				
Histidine	7.10±0.01 ^a	5.90±0.01 ^c	6.49±0.02 ^b	2.00
Valine	5.55±0.02 ^c	8.10±0.01 ^b	8.49±0.01 ^a	4.30
Phenylalanine	4.80±0.02 ^c	6.23±0.04 ^a	5.88±0.03 ^b	-
Threonine	1.99±0.01 ^c	4.06±0.01 ^b	4.42±0.02 ^a	3.10
Methionine	0.08±0.01 ^c	0.14±0.01 ^b	0.39±0.01 ^a	2.70
Lysine	43.01±0.10 ^a	19.61±0.05 ^b	17.36±0.06 ^c	5.70
Isoleucine	2.94±0.04 ^c	5.35±0.02 ^b	5.38±0.02 ^a	3.20
Leucine	16.26±0.03 ^a	15.26±0.02 ^b	13.63±0.05 ^c	6.60
Non Essential amino acids				
Arginine	41.76±0.10 ^a	23.50±0.11 ^b	17.32±0.13 ^c	-
Proline	19.73±0.03 ^a	19.31±0.02 ^b	10.66±0.09 ^c	-
Serine	5.31±0.04 ^c	9.19±0.01 ^b	9.50±0.01 ^a	-
Glutamic acid	40.62±0.12 ^b	44.08±0.10 ^a	35.18±0.09 ^c	-
Glycine	5.03±0.01 ^b	5.19±0.03 ^a	4.75±0.02 ^c	-
Alanine	4.23±0.01 ^c	6.06±0.03 ^b	6.22±0.05 ^a	-
Tyrosine	0.71±0.01 ^c	1.37±0.01 ^b	1.58±0.01 ^a	5.20
Aspartic acid	12.63±0.15 ^c	33.25±0.22 ^b	56.18±0.18 ^a	-

The data are presented as the means±standard deviations (n=3). In each row, identical letters indicate statistically insignificant differences, whereas highlighted letters indicate statistically significant differences ($p \leq 0.05$) between ungerminated faba bean seeds and dark-germinated and traditional germinated seeds at the end of germination, according to Duncan's post hoc test

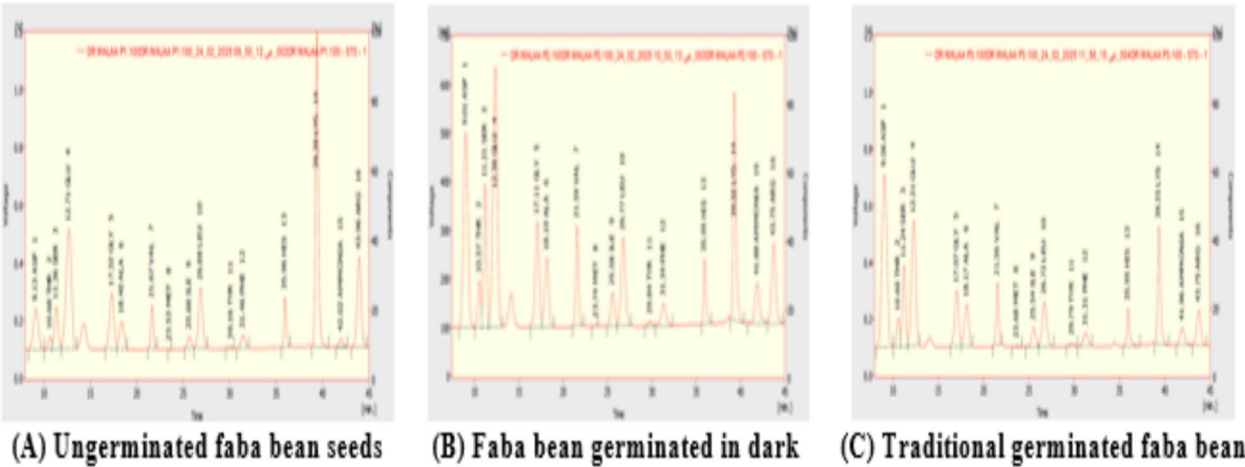


Fig. 3 Amino acid profile of the extracts of (A) ungerminated faba bean seeds, (B) Faba bean germinated in the dark at the end of germination and (C) traditional germinated faba bean at the end of germination

produced significant ($p < 0.05$) improvements, these effects were less pronounced, with GABA reductions of 1.27-fold, 1.33-fold, and 1.29-fold and AChE increases of 1.52-fold, 1.48-fold, and 1.48-fold in the cerebrum, cerebellum, and hippocampus, respectively.

Dopamine and serotonin: In all three brain regions, acrylamide exposure led to significant reductions in both

dopamine and serotonin levels. Dopamine decreased to approximately 1.4-fold greater than the control values, whereas serotonin substantially decreased to ~1.7-fold greater than the normal level. These neurotransmitters are crucial for mood and motor function. The germinated faba beans demonstrated a superior restorative capacity, reversing a substantial majority of the damage.

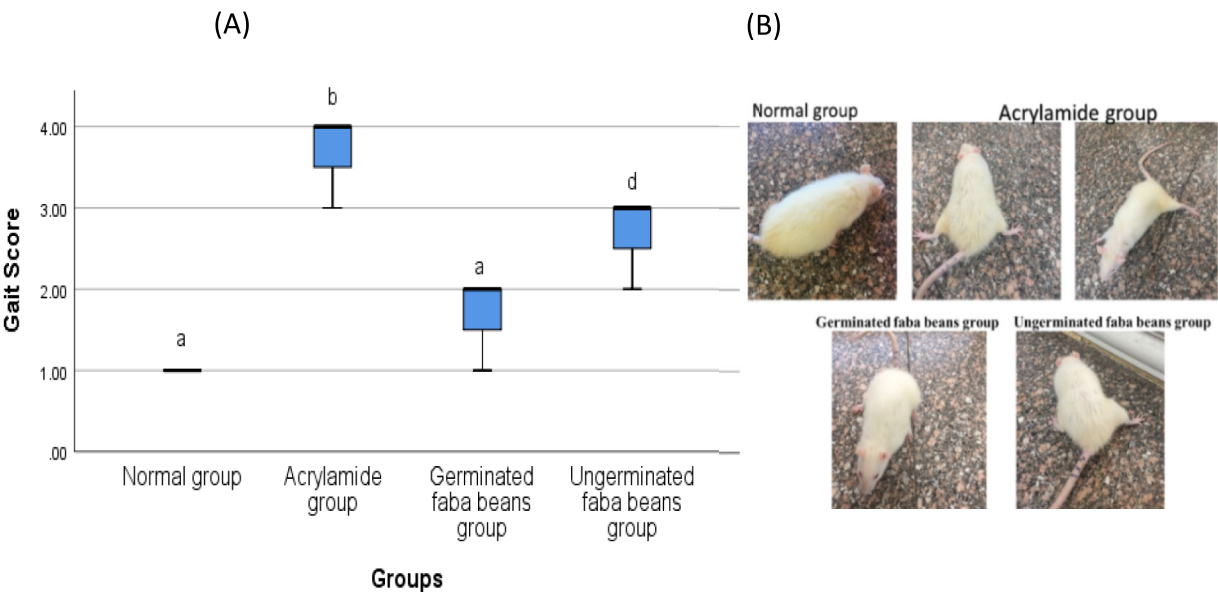


Fig. 4 The protective effects of germinated and ungerminated faba beans on gait in acrylamide-treated rats. **A** Box plot illustrating the gait scores among the different groups ($n=8/\text{group}$). Different letters (a, b, d) above the bars indicate a statistically significant difference between the groups ($p < 0.05$), whereas similar letters denote nonsignificant variation. **B** Representative photographs showing the physical posture and gait of the rats in each experimental group at the experimental endpoint

Table 4 Effects of germinated and ungerminated faba bean extracts on nutritional parameters, body weight, and brain weight in acrylamide-treated rats

Groups Parameters	Normal group	Acrylamide group	Germinated faba beans group	Ungerminated faba beans group
Initial body weight (g)	239.56 ± 6.42 ^a	239.50 ± 5.92 ^a	239.60 ± 6.14 ^a	239.54 ± 5.98 ^a
Final body weight (g)	270.10 ± 8.10 ^a	258.30 ± 7.12 ^b	268.50 ± 6.21 ^{ac}	263.30 ± 6.14 ^c
Body weight gain (g)	30.60 ± 2.11 ^a	18.80 ± 0.98 ^b	29.00 ± 1.57 ^a	23.80 ± 2.02 ^c
Total food intake (g)	232.80 ± 9.11 ^a	196.50 ± 7.20 ^b	220.10 ± 8.21 ^{ac}	211.40 ± 7.99 ^{bc}
Feed efficiency ratio	0.13 ± 0.012 ^a	0.10 ± 0.01 ^b	0.13 ± 0.01 ^a	0.11 ± 0.01 ^b
Brain weight (g)	1.95 ± 0.04 ^a	2.41 ± 0.03 ^b	2.01 ± 0.05 ^a	2.05 ± 0.04 ^a
Relative brain weight	0.72 ± 0.02 ^a	0.93 ± 0.01 ^b	0.75 ± 0.02 ^a	0.78 ± 0.02 ^d

The values are expressed as the means ± standard errors ($n=8$). Means within a row that share a common letter are not significantly different, whereas those with different letters are significantly different ($p < 0.05$), according to Duncan's post hoc test. The feed efficiency ratio was calculated as body weight gain divided by total food intake

Specifically, they restored 72.7% to 85.7% of dopamine and 83.3% to 85.4% of serotonin levels across the cerebrum, cerebellum, and hippocampus. In contrast, the ungerminated faba beans provided a significantly weaker protective effect, with only 32.0% to 47.1% dopamine deficit and 45.0% to 45.2% serotonin deficit. This consistent pattern across all brain regions highlights the significantly enhanced neuroprotective efficacy of the germination process.

Microscopic and immunohistochemical findings

Cerebrum

The control group exhibited a histological structure composed of six distinct layers, ranging from a superficial molecular layer containing axons and dendrites to deeper pyramidal layers. The underlying white matter consisted primarily of myelinated axons. In contrast, the acrylamide group demonstrated marked neuronal degeneration and increased neuronal cell death, particularly within the cortex. Degeneration of pyramidal neurons in the cerebral cortex is associated with vacuolisation

Table 5 Effects of germinated and ungerminated faba bean extracts on oxidative stress markers (MDA, GSH, SOD, NO) and inflammatory Cytokines (IL-6, TNF- α) in various brain regions of acrylamide-treated rats

Brain tissues	Groups Parameters	Normal group	Acrylamide group	Germinated faba beans group	Ungerminated faba beans group
cerebrum	MDA (nmol/g)	5.18 $\pm$ 1.03 ^a	18.04 $\pm$ 0.95 ^b	8.52 $\pm$ 1.12 ^a	13.85 $\pm$ 1.14 ^d
	SOD (U/g)	78.67 $\pm$ 0.55 ^a	32.81 $\pm$ 0.34 ^b	72.47 $\pm$ 0.57 ^a	53.74 $\pm$ 0.58 ^d
	NO (nmol/g)	39.96 $\pm$ 0.79 ^a	98.31 $\pm$ 1.46 ^b	34.46 $\pm$ 0.64 ^a	67.38 $\pm$ 0.71 ^d
	GSH (μ mol/g)	21.80 $\pm$ 0.67 ^a	9.27 $\pm$ 0.58 ^b	16.78 $\pm$ 0.65 ^a	12.95 $\pm$ 0.56 ^d
	IL-6 (pg/g)	8.52 $\pm$ 0.65 ^a	35.22 $\pm$ 0.99 ^b	15.38 $\pm$ 0.76 ^a	25.3 $\pm$ 0.69 ^d
	TNF- α (pg/g)	10.84 $\pm$ 1.15 ^a	33.60 $\pm$ 0.99 ^b	16.32 $\pm$ 0.95 ^a	24.96 $\pm$ 1.23 ^d
cerebellum	MDA (nmol/g)	4.28 $\pm$ 1.12 ^a	15.54 $\pm$ 1.02 ^b	7.54 $\pm$ 0.95 ^{ac}	12.64 $\pm$ 1.11 ^c
	SOD (U/g)	75.82 $\pm$ 0.64 ^a	33.69 $\pm$ 0.55 ^b	69.65 $\pm$ 0.58 ^a	52.78 $\pm$ 0.60 ^d
	NO (nmol/g)	40.14 $\pm$ 0.68 ^a	88.9 $\pm$ 1.34 ^b	46.31 $\pm$ 0.59 ^a	63.71 $\pm$ 0.58 ^d
	GSH (μ mol/g)	23.85 $\pm$ 0.48 ^a	10.62 $\pm$ 0.44 ^b	18.58 $\pm$ 0.37 ^{ac}	14.6 $\pm$ 0.42 ^c
	IL-6 (pg/g)	8.47 $\pm$ 0.71 ^a	33.03 $\pm$ 0.87 ^b	13.94 $\pm$ 0.64 ^a	22.48 $\pm$ 0.75 ^d
	TNF- α (pg/g)	11.14 $\pm$ 1.32 ^a	36.76 $\pm$ 1.07 ^b	16.38 $\pm$ 1.21 ^a	25.56 $\pm$ 1.31 ^d
hippocampus	MDA (nmol/g)	3.49 $\pm$ 0.98 ^a	12.77 $\pm$ 0.75 ^b	6.32 $\pm$ 1.11 ^{ac}	9.45 $\pm$ 1.51 ^c
	SOD (U/g)	74.46 $\pm$ 0.77 ^a	31.02 $\pm$ 0.58 ^b	65.02 $\pm$ 0.67 ^a	50.13 $\pm$ 0.7 ^d
	NO (nmol/g)	39.06 $\pm$ 0.59 ^a	85.72 $\pm$ 1.52 ^b	33.11 $\pm$ 0.48 ^c	60.42 $\pm$ 0.55 ^d
	GSH (μ mol/g)	18.80 $\pm$ 0.56 ^a	7.31 $\pm$ 0.61 ^b	14.20 $\pm$ 1.12 ^{ac}	11.87 $\pm$ 1.22 ^c
	IL-6 (pg/g)	8.09 $\pm$ 0.68 ^a	29.93 $\pm$ 0.85 ^b	13.83 $\pm$ 0.72 ^{ac}	20.88 $\pm$ 0.69 ^c
	TNF- α (pg/g)	10.21 $\pm$ 1.52 ^a	32.67 $\pm$ 1.03 ^b	15.22 $\pm$ 1.14 ^a	23.04 $\pm$ 1.11 ^d

In each row, groups that share a common letter are not significantly different from each other ($p > 0.05$). Conversely, groups that do not share a common letter are significantly different from one another ($p < 0.05$), according to Duncan's post hoc test. The data are presented as the mean value $\pm$ standard error for each group, with each group containing eight rats. MDA and NO are expressed in nmol/g; SOD, in U/g; GSH, in μ mol/g; and IL-6 and TNF- α , in pg/g

of the neuronal cytoplasm and loss of cellular integrity. Axonal swelling and degeneration, likely resulting from disrupted axonal transport, were especially evident in the white matter, where myelinated axons were notably affected. Both astrocytes and microglia exhibited reactive changes in response to toxicity, with microglial activation observed. In the germinated faba bean group, these pathological changes were significantly ameliorated, as evidenced by only mild vacuolisation of the neuronal cytoplasm. The ungerminated faba bean group displayed microglial activation, vascular congestion, and

lymphocytic infiltration within the Virchow–Robin space (Fig. 5).

Immunohistochemical staining for GFAP was reported. In the control group, there was minimal reactivity (+). Exposure to acrylamide results in a strong increase in GFAP immunoreactivity in the cerebrum (+++), indicating astrocyte activation. In areas of neuronal damage or degeneration, GFAP staining markedly increased, particularly around regions where axons or neurons were affected. The germinated group showed minimal reactivity (+) that was the same as that of the control group.

Table 6 Effects of germinated and ungerminated faba bean extracts on neurotransmitter levels (GABA, AChE, Dopamine, Serotonin) in various brain regions of acrylamide-treated rats

Brain tissues	Groups parameters	Normal group	Acrylamide group	Germinated faba beans group	Ungerminated faba beans group
cerebrum	GABA ($\mu\text{g/g}$)	74.73 $\pm$ 3.85 ^a	171.73 $\pm$ 3.22 ^b	96.73 $\pm$ 4.32 ^a	135.78 $\pm$ 4.11 ^d
	AChE (U/g)	210.54 $\pm$ 4.76 ^a	91.52 $\pm$ 3.71 ^b	192.51 $\pm$ 3.92 ^a	141.02 $\pm$ 4.22 ^d
	Dopamine (ng/g)	524.21 $\pm$ 6.21 ^a	374.43 $\pm$ 5.29 ^b	488.21 $\pm$ 5.11 ^a	432.67 $\pm$ 5.81 ^d
	Serotonin (ng/g)	533.52 $\pm$ 7.05 ^a	310.17 $\pm$ 6.12 ^b	496.26 $\pm$ 4.98 ^a	411.09 $\pm$ 5.11 ^d
cerebellum	GABA ($\mu\text{g/g}$)	77.34 $\pm$ 4.04 ^a	174.15 $\pm$ 3.76 ^b	97.80 $\pm$ 4.26 ^a	130.97 $\pm$ 4.32 ^d
	AChE (U/g)	215.03 $\pm$ 5.12 ^a	100.48 $\pm$ 3.94 ^b	194.51 $\pm$ 4.51 ^a	148.34 $\pm$ 5.11 ^d
	Dopamine (ng/g)	520.72 $\pm$ 6.10 ^a	359.10 $\pm$ 4.88 ^b	476.72 $\pm$ 5.24 ^a	410.79 $\pm$ 5.54 ^d
	Serotonin (ng/g)	533.52 $\pm$ 5.43 ^a	310.17 $\pm$ 5.05 ^b	496.26 $\pm$ 4.56 ^a	411.09 $\pm$ 4.97 ^d
hippocampus	GABA ($\mu\text{g/g}$)	75.52 $\pm$ 3.72 ^a	169.76 $\pm$ 3.55 ^b	98.51 $\pm$ 3.80 ^a	131.13 $\pm$ 4.56 ^d
	AChE (U/g)	212.13 $\pm$ 5.06 ^a	99.04 $\pm$ 4.13 ^b	193.01 $\pm$ 4.80 ^a	146.9 $\pm$ 4.43 ^d
	Dopamine (ng/g)	522.13 $\pm$ 5.79 ^a	357.61 $\pm$ 5.32 ^b	498.60 $\pm$ 5.63 ^a	435.11 $\pm$ 6.03 ^d
	Serotonin (ng/g)	520.31 $\pm$ 5.79 ^a	307.87 $\pm$ 4.79 ^b	489.33 $\pm$ 5.13 ^a	403.47 $\pm$ 5.30 ^d

The values are expressed as the means $\pm$ standard errors ($n=8$). Means within a row that share a common letter are not significantly different, whereas groups that do not share a common letter are significantly different from one another ($p < 0.05$), according to Duncan's post hoc test. GABA: Gamma-aminobutyric acid; AChE: Acetylcholinesterase. Dopamine and serotonin are expressed in nJ/g; AChE in U/g; and GABA in $\mu\text{g/g}$

Less GFAP immunoreactivity (++) was detected in the ungerminated group (Fig. 5).

Caspase-3 staining was estimated. The control group showed no reactivity (-) to caspase-3. In contrast, the cerebral cortex displayed significant neuronal damage after acrylamide exposure, with caspase-3-positive cells (+++) mainly in the cortical layers, especially in regions of neuronal loss. Compared with the acrylamide group, the germinated group presented minimal staining (+), whereas the ungerminated group presented less immunoreactivity (++) (Fig. 5).

Cerebellum

In the control group, a distinctive three-layered structure was observed. The molecular layer contained Purkinje cell dendrites, granule cell axons, and parallel fibres. The second layer, the Purkinje cell layer, contained large Purkinje cells. The last layer, the granular layer, is rich in granule cells and Golgi cells and is densely packed with small neurons. In contrast, the acrylamide group presented degeneration of Purkinje cells that may have undergone necrosis, leading to a reduction in the number

of Purkinje cells within the Purkinje cell layer. The granule cell layer was also affected in this group. **Glios**is (astrogliosis) was detected, with hypertrophied astrocytes and reactive microglia being observed in response to Purkinje cell and granule cell death. Additionally, vacuolisation within the cerebellar tissue was observed, particularly in the molecular and granular layers. The germinated group, in comparison, showed improvements in all previous findings, including only mild vacuolisation within the cerebellar tissue. In the ungerminated group, gliosis and degeneration of Purkinje cells were detected (Fig. 6).

The immunohistochemical reaction of the control group to GFAP was minimal (+). Exposure to acrylamide led to a significant increase in GFAP expression in the cerebellum (+++). Reactive gliosis, as indicated by GFAP staining, can be observed around the Purkinje cell layer, where neurons are damaged or dying. Additionally, the granular layer of the cerebellum, which contains many small granule cells, may also show reactive gliosis and GFAP-positive astrocytes, especially in areas with neuronal loss. Staining was minimal (+) in the

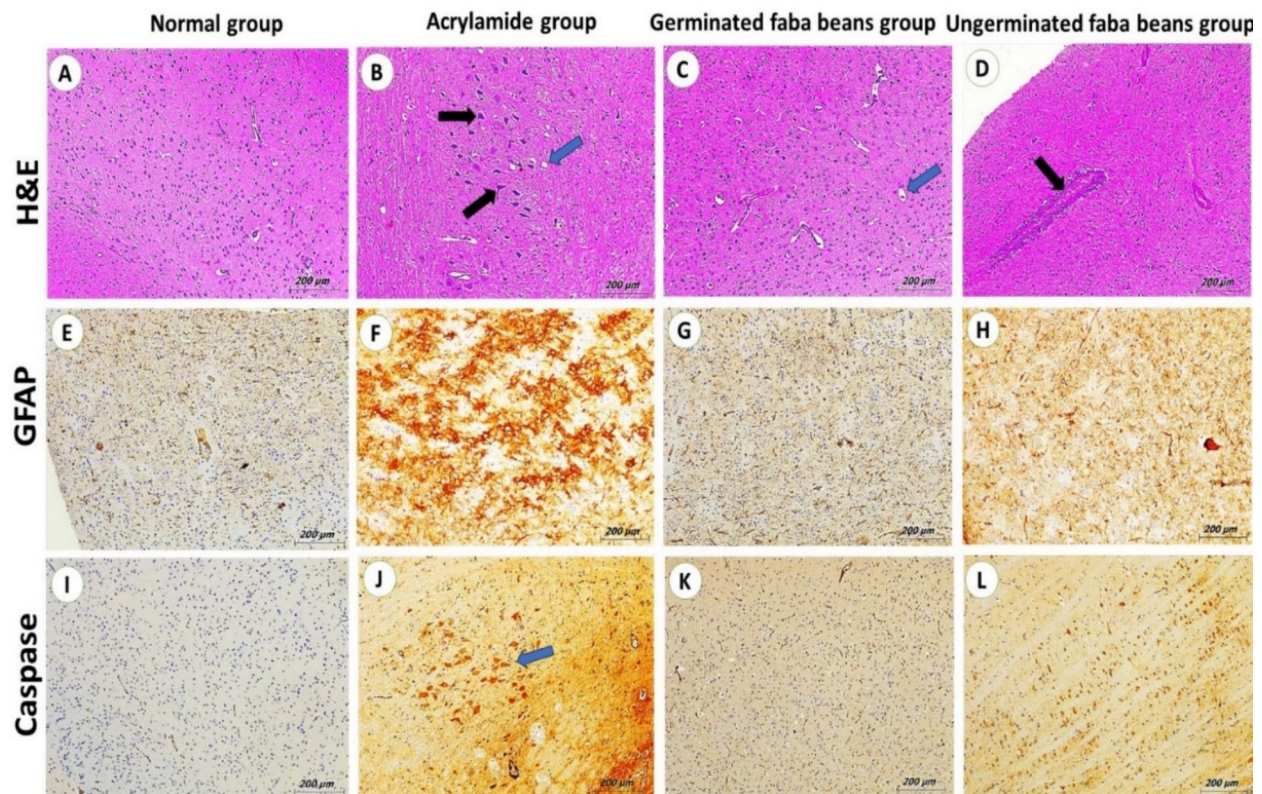


Fig. 5 Photomicrograph of a rat cerebrum experimentally exposed to acrylamide (scale bar, 200 µm). **A** Normal histological structure of the control group. **B** Increased gliosis reactivity (black arrows) and neuron degeneration with vacuolation (blue arrow). **C** Neuron degeneration with vacuolation (blue arrow). **D** Congested blood vessel with perivascular cuffing (black arrow). **E–H** Immunoreactivity to GFAP. **E** minimal reaction (+). **F** strong reaction (+++). **G** minimal reaction (+). **H** moderate reaction (++). **I–L** Immunoreactivity to caspase-3. **I** minimal reaction (+) **J** strong reaction (+++). **K** minimal reaction (+). **L** moderate reaction (++)

germinated group and moderate (++) in the ungerminated group (Fig. 6).

Immunohistochemical staining for caspase-3 revealed strong positivity (+++) in neurons that were undergoing programmed cell death due to the neurotoxic effects of acrylamide. Increased caspase-3 staining is likely observed in the Purkinje cell layer, where these neurons are undergoing apoptosis. Additionally, caspase-3 staining is likely to be observed in the granule cell layer of the cerebellum, particularly in regions with significant neuronal loss (acrylamide group). The control group showed no reactivity (-). The germinated group showed minimal reactivity (+) to caspase-3. A moderate reaction (++) in the ungerminated group was observed (Fig. 6).

Hippocampus

The control group was composed of the dentate gyrus (with granule cells), the Cornu Ammonis (CA) regions (containing pyramidal cells), and the subiculum (output region). Mild neuronal loss and neuron death, particularly in pyramidal cells, were detected in the acrylamide

group. In the other groups, there were no pathological alterations (Fig. 7).

Discussion

ACR is a well-known neurotoxic environmental contaminant that frequently forms in heat-processed foods via the Maillard reaction (Matoso et al., 2019). Given its established association with neurodegenerative effects, it has heightened public health concerns and stimulated research into protective strategies.

Previous studies have demonstrated that faba bean seed extracts contain high levels of polyphenols and exhibit significant antioxidant activity, both of which increase during sprouting (El-Mergawi & Taie, 2014; Okumura et al., 2016). In contrast, the present study is the first to systematically compare two germination methods to optimise sprout quality in the Egyptian cultivar Giza 843. Additionally, we investigated the neuroprotective and modulatory effects of germinated faba beans against acrylamide-induced neuroinflammation in a Sprague–Dawley rat model. The neurotoxic effects of acrylamide were assessed via the use of oxidative stress markers,

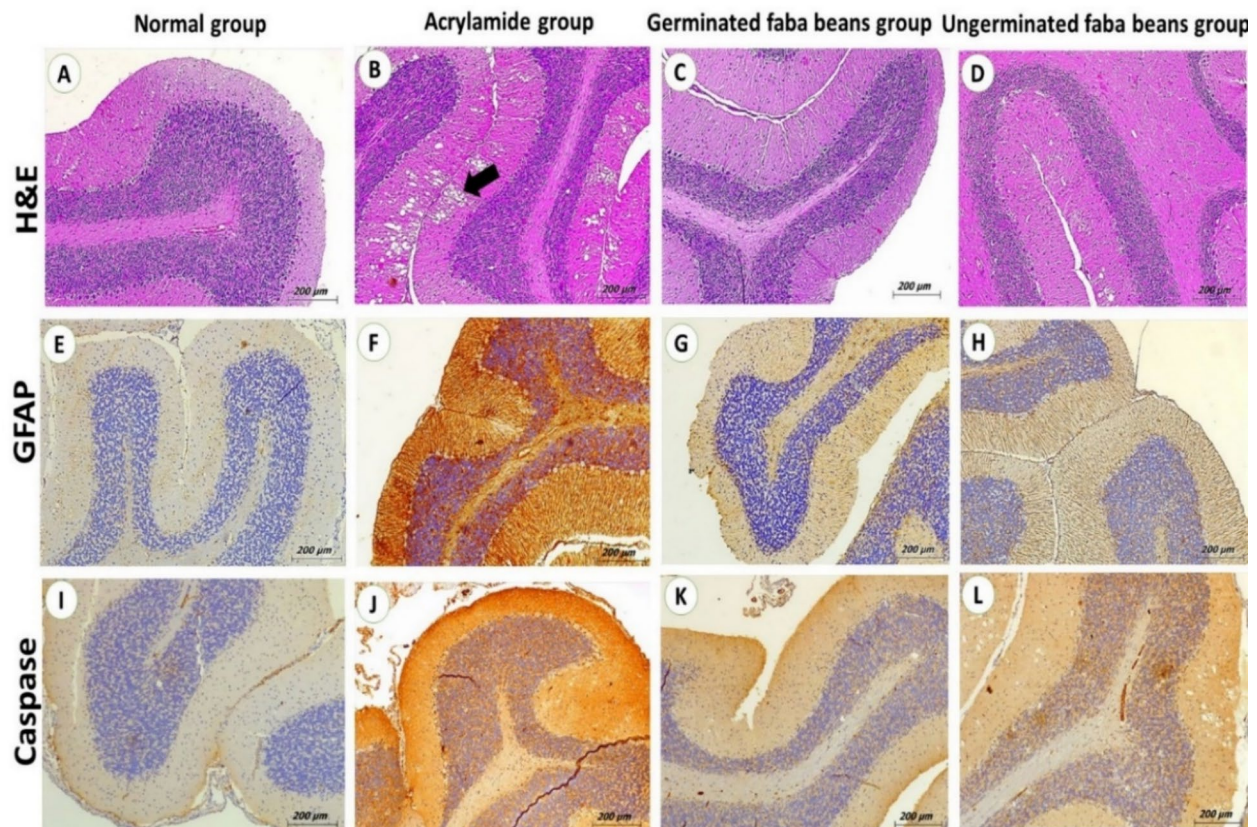


Fig. 6 Photomicrograph of a rat cerebellum experimentally exposed to acrylamide (scale bar, 200 μm). **A** Normal histological structure of the control group. **B** Neuron degeneration with vacuolation in the granular layer (black arrow). **C** normal histological structure. **D** mild neuron degeneration with vacuolation. **E–H** Immunoreactivity to GFAP. **E** minimal reaction (+). **F** strong reaction (+++). **G** minimal reaction (+). **H** moderate reaction (++). **I–L** Immunoreactivity to caspase-3. **I** minimal reaction (+). **J** strong reaction (+++). **K** minimal reaction (+). **L** moderate reaction (++)

inflammatory and neurotransmitter changes, histopathological analysis, and GFAP immunolocalisation in the cerebrum, cerebellum, and hippocampus.

Faba beans, particularly the Giza 843 cultivar, are rich sources of bioactive compounds, including phenolic compounds and flavonoids, with demonstrated antioxidant activity (Dhull et al., 2022; Mekky et al., 2020; Thakur & Thomas, 2024). Germination significantly increased ($P \leq 0.05$) these bioactive components, and germination significantly increased ($P \leq 0.05$) these bioactive components. The effects of germination were ranked as follows: traditional germinated > dark > ungerminated for phenolic and flavonoid contents and DPPH activity, confirming the method-dependent nature of these biochemical modifications (Mekky et al., 2020).

HPLC analysis revealed significant ($p < 0.05$) germination-induced changes in specific phytochemicals. The effects of germination were ranked as follows: traditional germinated > dark > ungerminated for phenolic and flavonoid compounds. In addition, traditional germinated plants generated novel compounds such as chlorogenic

acid (0.0005 mg/g), hesperidin (1.0661 mg/g) and kaempferol (0.8857 mg/g). These structural modifications likely contribute to the observed increase in antioxidant activity and may represent stress-response mechanisms (Khang et al., 2016).

Nutritional analysis revealed the dual effects of germination on amino acids: while valine (8.49 mg/g), threonine (4.42 mg/g), and methionine (0.39 mg/g) were increased through traditional germination, lysine (17.36 mg/g) and leucine (13.63 mg/g) were reduced. These method-specific alterations in protein quality may be due to the activity of proteolytic enzymes (Luo et al., 2014). Additionally, the fact that accumulated amino acids can be applied in other metabolic procedures, such as certain biochemical reactions or the synthesis of new proteins, may be the reason for the decrease in the content of some amino acids (Ospankulova et al., 2025). Consistent with the findings of Kuo et al. (2004), these findings complement the inherent nutritional value of faba beans as rich sources of protein, fibre, and health-promoting phytochemicals (Mekky et al., 2020).

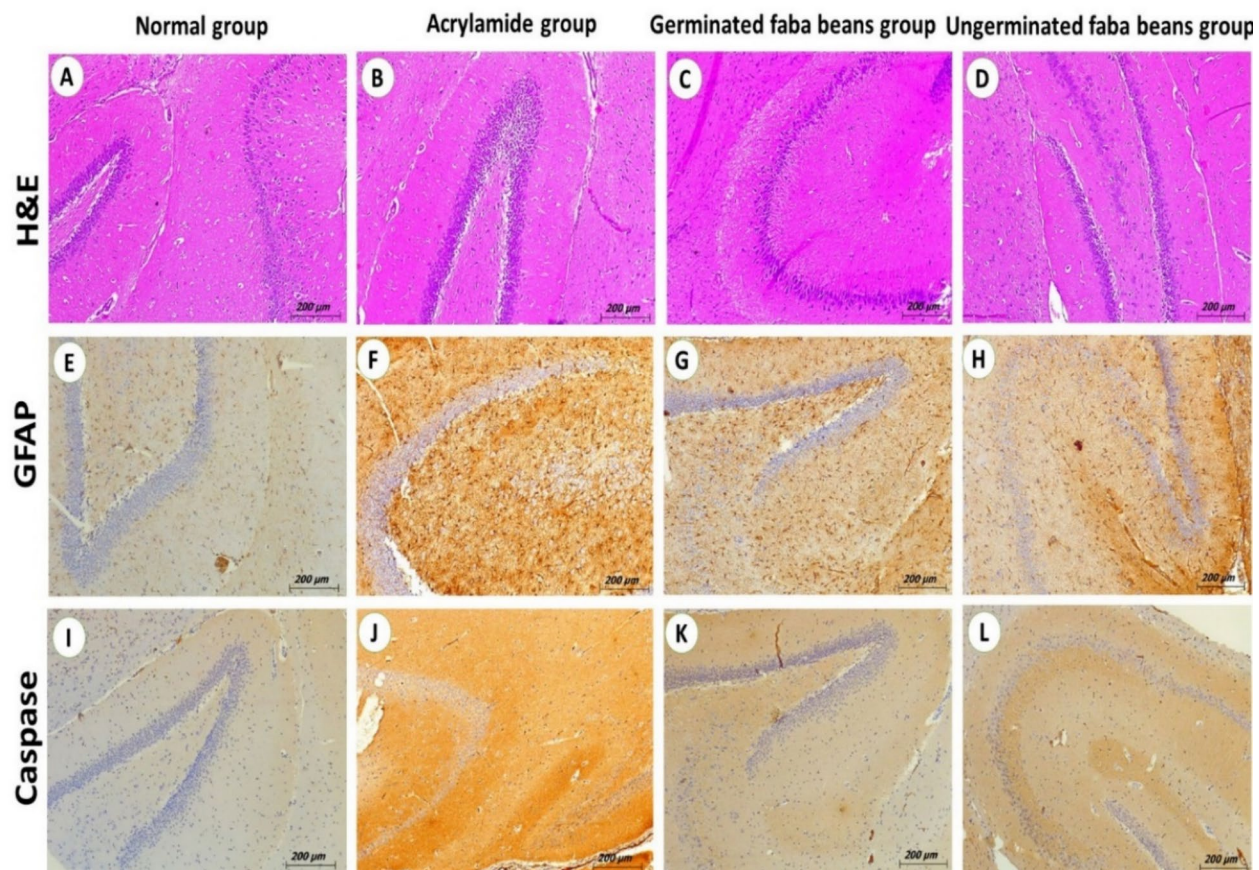


Fig. 7 Photomicrograph of a rat hippocampus experimentally exposed to acrylamide (scale bar, 200 μ m). **A** Normal histological structure of the control group. **B** Mild neuron degeneration. **C** normal histological structure. **D** mild neuron degeneration. **E–H** Immunoreactivity to GFAP. **E** minimal reaction (+). **F** strong reaction (+++). **G** minimal reaction (+). **H** minimal reaction (+). **I–L** Immunoreactivity to caspase-3. **I** no reaction (-). **J** strong reaction (+++). **K** minimal reaction (+). **L** minimal reaction (+)

The primary mechanism underlying ACR-induced neuroinflammation is oxidative stress (Erkekoglu & Baydar, 2014). ACR disrupts cellular redox homeostasis by increasing reactive oxygen species and nitric oxide synthase levels, leading to lipid peroxidation and the accumulation of malondialdehyde and nitric oxide. Concurrently, it depletes glutathione and inhibits antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Erkekoglu & Baydar, 2014). This oxidative damage extends to intracellular proteins and lipids, triggering neuronal death and synaptic failure (Kopanska et al., 2018).

These data collectively demonstrate that acrylamide induces significant neurotoxicity, characterised by a cascade of oxidative stress and inflammatory responses across multiple brain regions (cerebrum, cerebellum, and hippocampus). This is evidenced by the dramatic increase in the levels of pro-oxidant and proinflammatory markers and the simultaneous depletion of endogenous antioxidants. This finding is consistent with established research

on the mechanism of action of acrylamide, which highlights its role in inducing oxidative stress and biochemical perturbations in rat brains (Yousef & El-Demerdash, 2006). Furthermore, Zhao et al. (2022) reported this neurotoxicity, confirming that acrylamide triggers inflammation and neuronal damage.

ACR neuroinflammation is driven primarily by oxidative stress, inflammation, and disturbances in neurotransmitter function. A significant study involving rats exposed to ACR at a dosage of 50 mg/kg for 11 days revealed notable increases in critical oxidative stress markers, including malondialdehyde (MDA) and nitric oxide. Additionally, researchers have reported a substantial reduction in glutathione levels, as well as decreased activity of vital antioxidants such as superoxide dismutase and catalase, in the cerebrum, cerebellum, and hippocampus. These findings corroborate earlier studies that established a strong link between ACR exposure and oxidative stress, resulting in antioxidant depletion (Elhelaly et al., 2019; Essa et al., 2025a, b; Lai et al., 2017).

The significant increase in MDA levels, indicative of pronounced lipid peroxidation, effectively underscores the extent of free radical-mediated damage to neural tissues caused by ACR.

Inflammation significantly amplifies the neuronal damage caused by ACR (acrylamide). Research has demonstrated that ACR administration markedly elevates the levels of proinflammatory cytokines, such as IL-6 and TNF- α , which aligns with the critical findings of Elblehi et al. (2020). This potent inflammatory response, coupled with oxidative stress, drives profound neuronal degeneration and severe motor impairments. Compelling evidence is observed in the notably increased gait scores and the onset of hind limb paralysis in rats subjected to ACR treatment (LoPachin & Gavin, 2012). This highlights the urgent need to address inflammation as a key player in ACR-induced neurotoxicity.

The remarkable protective effect of germinated faba beans is a key finding of this study. The germination process likely enhances the bioavailability of phytochemicals, such as flavonoids and polyphenols, which are known for their potent antioxidant and anti-inflammatory properties (Li et al., 2025). These compounds may directly scavenge free radicals, upregulate endogenous antioxidant enzymes, and inhibit proinflammatory pathways. This dual mechanism of action could explain why germinated faba beans were so effective at reversing acrylamide-induced damage, a finding supported by other studies on the neuroprotective effects of faba bean extracts (Abdel-Sattar et al., 2020).

This compelling study underscores the impressive neuroprotective properties of germinated faba bean extract, driven by its significantly increased levels of polyphenols and antioxidants that are amplified during the germination process (Shetty et al., 2006). Notably, the phenolic compounds, flavonoids, and amino acids of the Giza 843 cultivar markedly increased following germination. Compared with ungerminated seeds, extracts derived from germinated beans demonstrated a superior ability to combat oxidative stress, reduce inflammatory cytokines, and restore neurotransmitter balance. These findings strongly align with the research of McGeer and McGeer (2004), who documented the potent anti-inflammatory effects of faba bean sprout extract in mice, further validating the promising therapeutic potential of the extract.

While ungerminated faba beans also provided some level of protection, their effects were less pronounced. This difference highlights the importance of the germination process in unlocking the full therapeutic potential of the faba bean. It has been shown that germination significantly impacts the phytochemical content and antioxidant activity of legumes, often leading to an increase in

these beneficial compounds (Anaemene & Fadupin, 2022; Elbadrawy & Mostafa, 2024).

Germination enhances both essential and nonessential amino acids, including aspartic acid, threonine, serine, alanine, methionine, and tyrosine. These amino acids are crucial for synthesising neurotransmitters and maintaining neuronal health (Khang et al., 2016; Vann et al., 2020). For example, tyrosine is a precursor for dopamine, whereas methionine aids in the production of glutathione, which increases antioxidant capacity (Yonar, 2011). The increased levels of these amino acids in germinated faba beans likely contributed to the neurochemical improvements observed in the rats treated with ACR.

The findings of this study provide clear evidence of the profound neurotoxic effects of acrylamide, which extend beyond oxidative damage to include severe dysregulation of key neurotransmitter systems. The observed increase in GABA and decrease in AChE activity in the acrylamide group was particularly significant. GABA is the primary inhibitory neurotransmitter, whereas AChE breaks down the excitatory neurotransmitter acetylcholine. An increase in GABA and a decrease in AChE could result in an overall shift towards neuronal inhibition, which may contribute to the neurological deficits associated with acrylamide toxicity, such as tremors and muscle weakness. This finding aligns with existing research showing that acrylamide can disrupt neurotransmitter synthesis and release, leading to neuronal dysfunction (Zhao et al., 2022).

A decrease in dopamine and serotonin levels is also a critical finding. These monoamines are integral to motor control, reward systems, and mood regulation. Their depletion has been linked to various neurological disorders, including Parkinson's disease and depression. The ability of acrylamide to deplete these neurotransmitters points to its widespread impact on central nervous system function (Abdel-Sattar et al., 2020). Germination enhances both essential and nonessential amino acids, including aspartic acid, threonine, serine, alanine, methionine, and tyrosine. These amino acids are crucial for synthesising neurotransmitters and maintaining neuronal health (Khang et al., 2016; Vann et al., 2020). For example, tyrosine is a precursor for dopamine, whereas methionine aids in the production of glutathione, which increases antioxidant capacity (Yonar, 2011). The increased levels of these amino acids in germinated faba beans likely contributed to the neurochemical improvements observed in the rats treated with ACR.

The modulatory effects of germinated faba bean extract are attributed to its high content of polyphenols and flavonoids, including diosmin, apigenin, and rutin. These compounds exhibit strong antioxidant and anti-inflammatory properties (Khang et al., 2016; Vann et al., 2020).

Diosmin, in particular, has been shown to suppress proinflammatory cytokines such as IL-6 and TNF- α (Shalkami et al., 2018), which parallels the reductions in cytokines observed in this study. Additionally, apigenin and rutin play a role in modulating the GABA and dopamine systems while enhancing antioxidant defenses (Mohammadkhanizadeh et al., 2025; Zhang et al., 2023).

ACR-induced neurotransmitter disruption was another critical aspect of this study. ACR exposure significantly reduced serotonin (SER) and dopamine (Dp) levels, which is consistent with the findings of Husain et al. (2012). This decline may result from the interaction of ACR with tyrosine and tryptophan, the precursors for Dp and SER synthesis, as well as increased monoamine oxidase activity, which accelerates neurotransmitter breakdown (Binder & Scharfman, 2004). Furthermore, ACR impaired AChE activity, likely through free radical formation and synaptic dysfunction (Lakshmi et al., 2012; LoPachin et al., 2004). Cotreatment with germinated faba bean extract restored AChE activity, highlighting its antioxidant properties.

GABA, a key inhibitory neurotransmitter, also plays a role in ACR-induced neuroinflammation. ACR treatment increased brain GABA levels, likely due to its toxic effects on neurotransmitter balance (Aboubakr et al., 2019; Tian et al., 2015). The elevated GABA levels in the treated groups may explain the observed improvements in neurochemical balance and movement disorders.

The most compelling aspect of this study is the potent neuroprotective effect of germinated faba beans. The data clearly revealed that these compounds not only counteract oxidative stress and inflammation but also restore neurotransmitter levels, indicating a comprehensive protective mechanism. This could be attributed to the increased bioavailability of bioactive compounds such as L-DOPA (a precursor to dopamine) and tryptophan (a precursor to serotonin), along with potent antioxidants and anti-inflammatory agents present in germinated faba beans. Germination is known to increase the concentration and bioavailability of these beneficial compounds, making them more effective than ungerminated beans (Fuentes-Herrera et al., 2023).

In contrast, while the ungerminated faba beans provided some benefit, their less pronounced effects confirm that germination is a crucial step in unlocking the full therapeutic potential of this legume.

Our study investigated the histopathological effects of acrylamide exposure on the brain, focusing specifically on the cerebrum, cerebellum, and hippocampus. In the control group, the brain structures remained intact, and no significant damage was observed in any of these regions. However, acrylamide exposure results in severe neurotoxic effects across all brain areas, providing strong

evidence of its potential as a neurotoxin (Abo-Aziza et al., 2022; Farouk et al., 2021; Jangir et al., 2016). The most significant damage occurred in the cerebrum, where neuronal degeneration was evident, characterised by cell swelling, vacuolisation, and loss of neurons. Additionally, glial activation was prominent, as indicated by increased GFAP expression, which reflects astrocytic gliosis in response to neuronal injury, as previously described by Ahrari Roodi et al. (2018) and Elsayed et al. (2024).

The group that received both germinated faba beans and acrylamide showed partial protection from the neurotoxic effects of acrylamide (Lai et al., 2017; Liu et al., 2021). This group provides valuable insight into the potential protective effects of germinated faba beans. The microscopic findings suggest that germinated faba beans contain protective compounds, such as antioxidants, that may mitigate some of the harmful effects of acrylamide. This finding aligns with previous studies that have suggested that germinated plants may have enhanced antioxidant properties, which could counteract the oxidative stress caused by acrylamide exposure (Guo et al., 2020). In the group exposed to ungerminated faba beans along with acrylamide, the protective effects were less pronounced than those in the germinated group.

Immunohistochemical staining for GFAP and Caspase-3 provides valuable insights into neuroinflammatory and neurodegenerative processes following acrylamide toxicity and the potential protective effects of germinated and ungerminated faba beans (Dasari et al., 2018). The comparison of these markers across the four experimental groups helps elucidate how acrylamide induces brain injury and how faba beans can potentially mitigate this damage. The immunohistochemical results indicate that acrylamide exposure induces significant neuroinflammation (indicated by GFAP staining) and neuronal apoptosis (indicated by caspase-3 staining). Compared with the acrylamide-only group, the germinated faba beans provided the most significant protection against these effects, with reduced astrocyte activation and lower neuronal apoptosis. These findings highlight the potential of germinated faba beans as neuroprotective agents against acrylamide-induced neuroinflammation (Farouk et al., 2021). The ungerminated faba beans also provided some level of protection, but their effects were weaker, supporting the idea that germination enhances the neuroprotective properties of faba beans.

Study limitations: While this study contributes valuable preliminary evidence, several limitations must be acknowledged. The experimental design utilised an acute exposure model, which may not adequately reflect the modulatory effects that could arise from chronic exposure to acrylamide. This limitation highlights the need for further research to fully understand the long-term

effects of acrylamide exposure on neurological health. The research was conducted using a single faba bean cultivar, and the observed effects may vary across different cultivars. The study did not incorporate advanced molecular techniques (such as transcriptomic or proteomic analyses) that could provide deeper mechanistic insights into the neuroprotective pathways involved. Additionally, while the *in vivo* results are promising, the translational potential for human applications remains to be established through clinical trials examining bioavailability, optimal dosing regimens, and long-term safety profiles. Future research is needed to determine the optimal dietary intake level and bioavailability of the active compounds in humans. These limitations highlight important directions for future research while not diminishing the current findings' significance.

Despite these limitations, we can provide a perspective on human relevance by estimating a dietary equivalent dose on the basis of our animal study. While we cannot yet make definitive recommendations, our findings offer a preliminary reference. The study used a faba bean extract dose of 300 mg/kg/day in rats. The standard body surface area conversion method results in a human equivalent dose (HED) of approximately 48.6 mg/kg/day. For a 70 kg adult, this would equate to a daily intake of approximately 3.4 g of the extract. While this precise dosage is for the experimental context and requires clinical validation, incorporating a meaningful amount of germinated faba beans into the diet could be a feasible strategy. Future research is necessary to determine the optimal dietary intake, bioavailability in humans, and long-term safety.

Conclusion

This study demonstrated that germination enhances the neuroprotective potential of the Egyptian faba bean cultivar Giza 843 against acrylamide-induced neurotoxicity. These data indicate that the extract from germinated faba beans ameliorates acrylamide-induced damage by reducing oxidative stress, attenuating neuroinflammation, and restoring neurotransmitter balance.

The protective effects were more pronounced in traditionally germinated beans than in ungerminated seeds, which was correlated with the observed increase in phenolic compounds, flavonoids, and specific amino acids during germination. Histopathological and immunohistochemical analyses provided supporting evidence that the germinated bean extract helped preserve neuronal structure and reduce the expression of markers of apoptosis and glial activation.

These findings position germinated faba beans as promising functional foods or therapeutic agents for

mitigating the neurotoxic risks associated with acrylamide exposure. Future research should focus on identifying the specific bioactive compounds responsible for these effects and evaluating their efficacy in other models of neurodegeneration.

Abbreviations

ABC	Avidin–biotin complex (immunohistochemistry technique)
AChE	Acetylcholinesterase
ACR	Acrylamide
AlCl ₃	Aluminum Chloride
CA1 & CA3	Cornu Ammonis regions of the hippocampus
CAT	Catalase
DAB	3,3'-Diaminobenzidine tetrahydrochloride (chromogen for immunohistochemistry)
DAD	Diode array detector (used in HPLC)
Dp	Dopamine
DPPH	2,2-Diphenyl-1-picrylhydrazyl (a free radical used in antioxidant assays)
ELISA	Enzyme-Linked Immunosorbent Assay
GABA	Gamma-Aminobutyric Acid
GFAP	Glial fibrillary acidic protein (a marker for astrocytes)
GPx	Glutathione Peroxidase
GSH	Glutathione
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
i.p.	Intraperitoneal (injection)
IL-6	Interleukin-6
MDA	Malondialdehyde (a marker of lipid peroxidation)
NO	Nitric oxide
P.O.	Per os (oral administration)
SER	Serotonin
SOD	Superoxide dismutase
TNF-α	Tumor necrosis factor-alpha

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Non.

Authors' contributions

****Hend A. Essa****: Proposed the main research point and designed the study, conducted the animal experiments, performed biochemical analyses, and carried out their statistical evaluation. Wrote the main draft and the final version of the manuscript and editing the manuscript. ****Walaa M. El Sayed****: Responsible for the methods, results, and analysis of the plant extracts. Wrote the manuscript. ****Ahmed Fotouh****: Responsible for the methods, results, and analysis of histopathological and immunohistochemical evaluations. Wrote the manuscript. All authors contributed to reviewing the manuscript. All authors have approved the manuscript for submission.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate

The study was approved by the General Organization for Teaching Hospitals and Institutes (GOTHI) Ethics Committee (Approval No.: IN000147) and complied with the National Institutes of Health guidelines for the care and use of laboratory animals (Garber et al., 2011).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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