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Effect of intermittent fasting on behavioral, biochemical and histopathological parameters in rats under Acrylamide exposure

Efecto del ayuno intermitente sobre los parámetros conductuales, bioquímicos e histopatológicos en ratas expuestas a acrilamida

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ABSTRACT

Acrylamide is a known neurotoxic and potentially carcinogenic compound. It remains as a major public health concern due to its widespread presence in heat-processed foods. Despite extensive research on acrylamide-induced toxicity, effective dietary strategies to mitigate its harmful impact remain limited. Intermittent fasting has recently emerged as a promising metabolic intervention shown to enhance cellular stress resistance and improve antioxidant capacity. This study was designed to investigate the effects of intermittent fasting on acrylamide-induced toxicity in rats. Wistar rats were randomly divided into four groups: control, acrylamide, intermittent fasting, and acrylamide + intermittent fasting. Intermittent fasting was applied every other day, while acrylamide was administered intraperitoneally at a dose of 30 mg/kg/day, three times per week. On day 28, behavioral assessments were performed using the Elevated Plus Maze, Open Field Test, hotplate, and rotarod tests. Biochemical analyses were conducted on blood samples, and oxidative stress parameters Catalase, Glutathione peroxidase, Superoxide dismutase were measured in liver, kidney, and brain tissues. Histopathological evaluations were also carried out. Histopathological findings indicated tissue damage in the acrylamide group and partial improvement in the acrylamide + intermittent fasting group. In the rotarod test, performance of the acrylamide + intermittent fasting group was similar to the control group, suggesting a protective effect. Catalase, Glutathione peroxidase, and Superoxide dismutase levels showed partial amelioration in kidney and brain tissues due to intermittent fasting. The results suggest that intermittent fasting may exert a protective effect against acrylamide-induced oxidative stress and behavioral impairments in rats. These findings highlight the potential of intermittent fasting as a non-pharmacological strategy to mitigate acrylamide toxicity.

Key words: Acrylamide, intermittent fasting, behavior, oxidative stress

RESUMEN

La acrilamida es un compuesto neurotóxico y potencialmente cancerígeno conocido. Sigue siendo un importante problema de salud pública debido a su amplia presencia en alimentos procesados térmicamente. A pesar de la extensa investigación sobre la toxicidad inducida por acrilamida, las estrategias dietéticas eficaces para mitigar su impacto nocivo siguen siendo limitadas. El ayuno intermitente se ha convertido recientemente en una prometedora intervención metabólica que ha demostrado mejorar la resistencia celular al estrés y la capacidad antioxidante. Este estudio se diseñó para investigar los efectos del ayuno intermitente sobre la toxicidad inducida por acrilamida en ratas. Las ratas Wistar se dividieron aleatoriamente en cuatro grupos: control, acrilamida, ayuno intermitente y acrilamida + ayuno intermitente. El ayuno intermitente se aplicó día por medio, mientras que, la acrilamida se administró por vía intraperitoneal a una dosis de 30 mg/kg/día, tres veces por semana. En el día 28 se realizaron evaluaciones conductuales mediante el Laberinto en Cruz Elevado, la Prueba de Campo Abierto, la prueba de placa caliente y la prueba de rotarod. Se efectuaron análisis bioquímicos en muestras de sangre, y se midieron parámetros de estrés oxidativo —Catalasa, Glutatión peroxidasa, Superóxido dismutasa— en hígado, riñón y tejido cerebral. También se llevaron a cabo evaluaciones histopatológicas. Los hallazgos histopatológicos indicaron daño tisular en el grupo acrilamida y una mejora parcial en el grupo acrilamida + ayuno intermitente. En la prueba de rotarod, el rendimiento del grupo acrilamida + ayuno intermitente fue similar al del grupo control, lo que sugiere un efecto protector. Los niveles de Catalasa, Glutatión peroxidasa y Superóxido dismutasa mostraron una mejora parcial en riñón y cerebro debido al ayuno intermitente. Los resultados sugieren que el ayuno intermitente podría ejercer un efecto protector contra el estrés oxidativo y las alteraciones conductuales inducidas por acrilamida en ratas. Estos hallazgos resaltan el potencial del ayuno intermitente como una estrategia no farmacológica para mitigar la toxicidad de la acrilamida.

Palabras clave: Acrilamida, ayuno intermitente, conducta, estrés oxidativo

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INTRODUCTION

Acrylamide is a chemical formed in certain conditions and unintentionally consumed with food. There are increasing number of publications which study effect of acrylamide in nervous system, blood viscosity, cancer or nutrition [1]. In order to model different exposure schemes of acrylamide on laboratory animals different dosages and protocols are chosen [2 , 3 , 4]. Intermittent fasting (IF), which involves restricting or completely abstaining food for certain periods, has recently become a widely studied subject due to its positive effects on health [5].

Among the most well-known positive effects of IF are weight loss and reduction of adipose tissue [6], increased insulin sensitivity, control of blood glucose levels, dyslipidemia, altered blood pressure as well as a reduction in metabolic syndrome [7 , 8]. Additionally, its contribution to cellular repair processes positively affects the health of many tissues, including the brain tissue. It has been shown to attenuate neuroinflammation and increase certain molecules such as brain-derived neurotrophic factor (BDNF) in brain tissue [9].

Various human studies have demonstrated the slowing of disease progression in Alzheimer's disease, epilepsy as well as multiple sclerosis through IF. Its effects on Parkinson's disease, autism spectrum disorder, and anxiety are mostly studied through animal research, with promising findings [10 , 11].

Intermittent fasting can help the body detoxify and eliminate metabolic waste. It is known to reduce the effects of exposure to harmful chemicals on the body. Although some animal studies have shown promising results in reducing the side effects of chemotherapeutics, its effectiveness in cancer treatment is controversial due to the potential adverse effects of fasting in cancer patients [12].

Evaluating the effects of IF on stress and anxiety through human studies can be challenging when considering individuals' beliefs, especially with religious fasts. Nonetheless, a meta-analysis conducted in 2021 involving 1,436 patients indicated that symptoms of stress, anxiety, and depression decreased in individuals after Ramadan fasting compared to control groups [13].

Conversely, a 2023 meta-analysis that excluded the influence of religious beliefs found no significant differences in anxiety and mood changes, although there was a decrease in depression scale scores [14]. Considering the diverse outcomes in the literature aim of this study is to investigate the potential protective effects of IF on behavioral, biochemical, histological, and oxidative stress parameters during acrylamide exposure in male rats (*Rattus norvegicus*).

MATERIALS AND METHODS

Twenty-eight male Wistar-albino rats, aged 4-8 weeks and weighing between 200-250 grams (g) (Mettler PM600), were used. During the acclimitization, the rats were provided with water and food *ad libitum* without restriction. To ensure standard conditions prior to the study, all rats were given the same standard pellet food, water, and care conditions (temperature: 20 ± 2 ºC, 12-hour light/dark cycle).

Then the animals were randomly assigned to four groups (n = 7 in each groups). The experimental procedures was carried out over 4 weeks. All administrations were conducted after an ethical approval from Hacettepe university Animal Experiments Ethics Committee (decision number= 2020/11-09). Control Group (C): Intraperitoneal (i.p.) saline injections were given three times a week. Acrylamide Group (AC): Rats received AC at a dose of 30 mg/kg/day (d) i.p. on three non-consecutive d per week (e.g. Monday, Wednesday and Friday), for a total duration of 4 weeks (12 injections; cumulative dose: 360 mg/kg).

The IF Group: Was applied to the rats every other d (24 h fasting / 24 h feeding). Acrylamide+Intermittent Fasting Group (ACIF): 30mg/kg/d i.p. acrylamide was given three times a week+intermittent fasting was applied every other d.

After the experimental procedures, Open Field Test and Elevated Plus Maze anxiety tests as well as rotarod and hot plate tests were conducted in the given order. In the literature different exposures are given to animals [2]. Over the 28-d experimental period, this protocol resulted in a cumulative acrylamide dose of 360 mg/kg (3 injections/week × 30 mg/kg × 4 weeks).

Behavioral tests

All behavioral tests were performed on d 28 of the experimental protocol. The IF schedule was arranged to ensure that all animals—whether in the IF or non-IF groups—were tested on a morning following a non-fasted night, minimizing potential confounding effects of acute fasting. Food was available *ad libitum* on d 1, removed on d 2 morning, and reintroduced on d 3 morning, continuing in a 24-h fasting / 24-h feeding cycle throughout the 28-d protocol. Thus, behavioral tests were conducted on the morning of d 28, when both IF and C groups had food available overnight.

On d 28, all animals underwent behavioral testing in the following fixed order: Open Field Test → Elevated Plus Maze → Rotarod → Hot Plate Test. All tests were performed during 09:00–12:00.

Open field test

Locomotor activity and anxiety-related behavior were assessed with open field test. Time spent in the center and perimeter area, the number of rearing events, and the number of grooming behaviors was recorded (5 min) in an open field square box with a defined perimeter and center. The apparatus was wiped with 70 % ethanol after each animal trial.

Elevated plus maze test

Following Open Field Test, rats were placed in the center of the plus-shaped maze facing an open arm. The number of entries into the open and closed arms, the time spent in these arms, and the percentages were evaluated in an elevated plus maze (5 min). An increase in the time and percentage spent in the closed arm was considered an indicator of increased anxiety. The apparatus was wiped with 70 % ethanol after each animal trial.

Rotarod test

Following elevated plus maze test, Rotarod test was conducted to assess locomotor activity in the rats in which, the duration of the rats on a rod rotating at 15 rpm was recorded. Following a habituation period three consecutive rotarod testing sessions were recorded and those recordings were included in the statistical analyses.

Hotplate test

The rats were placed on a platform heated to 52 °C, and the time to withdraw, lick or shake their paws due to the heat was recorded (Rotarod and hotplate devices were manufactured by the Hacettepe University researchers according to relevant literature).

Blood biochemistry

Immediately after completion of behavioral testing, animals were sacrified by high-volume blood collection under anesthesia, and measurements were made from the blood withdrawn into tubes. Relevant parameters; alkaline phosphatase (ALT), aspartate aminotransferase (AST), triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL), total cholesterol, creatinine, total protein, blood glucose, insulin were evaluated (Beckman coulter AU5800 and Unicel DXL800, USA) [15].

Oxidative stress analyses

Catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) were examined (Ransod SD-125, Ransel RS-504, Randox, UK) in the liver, kidney, and brain tissues of the rats. Relevant tissues were washed with 0.9 % NaCl and stored at -80 °C until analysis. Tissue samples (0.5 g) were homogenized in 5 mL of ice-cold homogenization buffer (1 mmol/L) using an Ultra Turrax T25 homogenizer (IKA, Staufen, Germany) and an ultrasonic homogenizer (20 KHz frequency, Bandelin Sonupuls). The buffer contained EDTA, 0.32 mol/L sucrose, and 10 nmol/L Tris-HCl, pH 7.4. The homogenization process lasted 8 min, followed by centrifugation at 10118 g (Hettich EBA 20 UK) for 30 min [16]. Procedures were carried out at 4 °C. The supernatants were used to determine the activity of antioxidant enzymes. The supernatants from liver, kidney, and brain tissue homogenates were used to evaluate the antioxidant enzymes SOD, glutathione peroxidase (GSH-Px), and CAT.

CAT Determination: CAT (EC 1.11.1.6) activity was measured at 240 nm wavelength using the method described by Aebi [17].

GPX Determination: Tissue GSH-Px (EC1.11.1.9) activity was measured at 340 nm wavelength according to the method of Paglia and Valentine [18].

SOD Determination: Tissue SOD (EC 1.15.1.1) activity was determined at 505 nm wavelength using the method of Sun et al. [19]. CAT, GPX and SOD was determined with Shimadzu UV-1800 UV-VIS Spectrophotometer, Kyoto, Japan.

Histopathological examination

Liver and kidney tissues were fixed in 10 % buffered formaldehyde solution for 72 h. The tissue samples were placed in an automatic tissue processor (Leica TP 1020, Leica Biosystems Nussloch GmbH, Nussloch, Germany) and underwent routine processing: dehydration through a series of alcohols (70, 80, 90 and 100 %), clearing through a series of Xylene, and embedding in paraffin. From these blocks, serial sections of 5 microns thick were taken using a microtome (Leica RM 2135, Leica TP 1020, Leica Biosystems Nussloch GmbH, Nussloch, Germany) and stained with routine Hematoxylin-Eosin (HE) staining technique. All stained slides were examined and photographed using a light microscope with a digital imaging system (Olympus DP12BSW, Tokyo, Japan)

Histopathological alterations in the liver and kidney tissues of each group (congestion, degeneration in hepatocytes, bile duct proliferation, and degeneration in tubular epithelium) were evaluated qualitatively by two pathologists according to relevant scoring systems [20 , 21].

The scoring system was as follows:

(-) score (negative score); no structural changes,

(+) score (1 positive score); mild degree,

(++) score (2 positive scores); moderate degree,

(+++) score (3 positive scores); severe structural changes.

Statistical analysis

SPSS 20 software was used for statistical analysis. Data are presented as mean ± standard deviation and $P < 0.05$ was considered significant. The analyzes were determined according to whether the data was parametric or not. The Shapiro–Wilk test was used to determine the normality of data distributions, while the variance homogeneity of groups was determined by the Levene test. Data were evaluated using the Kruskal-Wallis test. For comparisons, the Mann-Whitney-U test and Bonferroni correction was used.

RESULTS AND DISCUSSION

Protective effect of IF against acrylamide exposure was investigated through behavioral, oxidative stress, blood biochemical and histopathological analyses. Results from the examined parameters indicate that intermittent fasting exhibits a mitigating effect on the negative effects caused by acrylamide.

Weight monitoring of groups

The average weights on the first and last days of the experiment were as follows:

- C group: 229 g-266 g
- AC group: 230 g-238 g
- IF group: 224 g-214 g
- ACIF group: 225 g-195 g

Behavioral findings

Open field test

The number of unsupported rearing events was significantly lower in all groups (AC 3.43 ± 5.65, IF 2.43 ± 2.70, ACIF 2.0 ± 1.53) compared to the C group (12.29 ± 7.32).

Elevated plus maze

A significant difference was observed in the percentage of time spent in the closed/open areas between the AC (68.43 ± 41.58) and IF (7.86 ± 11.25) groups. The percentage of time spent in the closed/open arms was significantly higher in the AC group but not in ACIF group compared to the IF group. This indicates an increase in anxiety due to AC exposure and a partial protective effect induced by IF against AC.

Acrylamide is known to cause toxicity at both macro and cellular levels in the nervous system [22]. It causes oxidative damage, neuroinflammation as well as disrupting metabolism [23]. Its causes disturbance to the secretion of neurotransmitters, altering acetylcholinesterase activity [24].

On the other hand, evidence from animal studies suggests that IF reduces beta-amyloid accumulation, increases synaptic adaptations in the hippocampus, improves cognitive functions, reduce neurodegeneration, neuronal and cognitive damage in mice following traumatic brain injury or epilepsy [25 , 26 , 27]. It also augments BDNF, Neurotrophin 3 levels in rats thereby alleviating behavioral outcomes in some conditions such as diabetes [28]. Another protective effect of IF comes from decreasing oxidative stress and balancing authophagy mechanisms in certain conditions [29]. It is also suggested that IF also causes alleviation in the brain energy metabolism via ketone bodies [30].

Rotarod

The lowest value was observed in the AC group (16.1 ± 14.1s), and the highest value was observed in the IF group (50.7 ± 14.9s). In the C group, this value was found to be 47 ± 16.9s, while in the ACIF group, it was 23 ± 19.1s. The AC group walked for a significantly shorter time compared to the C and IF groups ($P < 0.05$).

Consistent with expectations [31], AC administration reduced walking time in the rotarod test in this current study. Fasting in the ACIF group partially reversed the decrease caused by AC, although not statistically significant. AC and its derivatives causes peripheral nerve damage and myelin loss which causes morphological damage to such nerves [32]. It also causes mitochondrial dysfunction and triggers necroptosis in Purkinje cells [33]. IF is known to have positive changes in mitochondrial function [34]. During IF some molecular markers are suggested such as BDNF, Gamma-Aminobutyric Acid, Growth Hormone/ Insulin-like Growth Factor-1, Fibroblast Growth Factor 2 and ketone bodies which end up with augmented resilience against cellular stress resistance and enhanced neurogenesis and synaptic plasticity [35]. The results observed in the present study may be linked to the findings reported in scientific literature.

Hot plate

The longest withdrawal time was observed in the AC group (11.7 ± 3.5s), while the shortest time was observed in the C (7 ± 1.7s). IF group had a time close to that of the C group (7.6 ± 3s). Although ACIF group (8.3 ± 3.2s) was lower than the AC group, there was no significant difference among the groups ($P > 0.05$).

Experimental procedure was set to 28 d in accordance with relevant literature [36]. Studies showed significant alterations in hotplate test due to acrylamide administration [37]. This unparallel results may be due to different experimental protocols.

Blood biochemistry

Blood cholesterol (HDL, LDL, total cholesterol (TCHOL)) and TG levels were reduced in IF and ACIF groups compared to C and AC which are significant in TG (C versus ACIF), TCHOL (C and AC versus ACIF), HDL (C and AC versus ACIF) and LDL (C and AC versus ACIF). A similar pattern is also present in total protein (TPROT) where AC have significantly higher value compared to IF and ACIF (TABLE I).

TABLE I
Blood biochemistry results

Groups	C	AC	IF	ACIF
HDL	41.00±6.87^{a}	41.57±8.60^{a}	30.71±6.47^{ab}	28.14±1.21^{b}
LDL	21.33±4.32^{a}	20.71±4.72^{a}	14.43±3.41^{ab}	13.00±1.15^{b}
CRE	0.18±0.02^{ab}	0.21±0.04^{a}	0.14±0.01^{b}	0.16±0.04^{ab}
TPROT	6.24±0.31^{ab}	6.33±0.41^{a}	5.73±0.35^{b}	5.78±0.27^{b}
TG	64.83±15.38^{a}	48.29±18.38^{ab}	43.43±11.47^{ab}	40.86±8.05^{b}
TCHOL	61.50±9.35^{a}	60.43±12.82^{a}	44.57±9.55^{ab}	41.57±1.62^{b}

C: Saline, AC: Acrylamide, IF: intermittent fasting, ACIF: acrylamide + intermittent fasting (n = 7 in each group). Different letters (a, b, c) indicate statistically significant difference. $P < 0.05$. HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein. CRE: Creatinine. TPROT: Total protein. TG: Triglyceride. TCHOL: Total cholesterol

These observed attenuations can be linked with every other day fasting. However increased creatinine (CRE) in AC can be due to effects of AC administration on kidney tissue. Since CRE value is not increased in ACIF as in AC it can be suggested that fasting may have protected kidneys agains harmful effects of AC exposure which is in parallel with histopathology. On the other hand no significant change in ALT and AST values needs to clarified since liver histopathology showed prominent impact of AC on liver.

Oxidative stress

Oxidative stress findings indicated a similar pattern in the brain, kidneys, and liver, showing comparable levels of CAT, GPX, and SOD enzymes. It was observed that the results, which were similar between the control and IF, decreased in the AC group, indicating that intermittent fasting either partially reversed or maintained this decrease at the same level, as seen in the ACIF group (TABLE II).

TABLE II
Results of the biochemical analyses of different oxidative parameters found in the liver, kidney, and brain tissues

	Biochemical Parameters	C	AC	IF	ACIF
Liver	CAT (EU/mg protein)	9.95 ± 1.54^{a}	5.25 ± 0.90^{b}	9.27 ± 1.69^{a}	5.49 ± 1.90^{b}
	SOD (EU/mg protein)	6448.57 ± 44.78^{a}	2746.31 ± 964.59^{b}	6516.00 ± 156.85^{a}	2733.05 ± 1615.77^{b}
	GPX (EU/mg protein)	0.21 ± 0.01^{a}	0.15 ± 0.02^{ab}	0.21 ± 0.04^{a}	0.14 ± 0.02^{b}
Kidney	CAT (EU/mg protein)	18.37 ± 3.12^{a}	11.04 ± 0.98^{b}	17.63 ± 2.36^{a}	14.10 ± 2.92^{ab}
	SOD (EU/mg protein)	1493.26 ± 131.46^{a}	1040.52 ± 70.92^{b}	1485.59 ± 274.88^{a}	1213.59 ± 144.06^{ab}
	GPX (EU/mg protein)	14.35 ± 1.40^{a}	9.83 ± 1.89^{b}	14.34 ± 2.09^{a}	10.63 ± 1.12^{b}
Brain	CAT (EU/mg protein)	65.56 ± 4.55^{a}	34.97 ± 4.78^{b}	58.83 ± 3.97^{a}	38.95 ± 5.39^{ab}
	SOD (EU/mg protein)	7159.33 ± 691.14^{a}	2740.39 ± 928.63^{b}	6736.57 ± 1083.74^{a}	2863.79 ± 499.14^{b}
	GPX (EU/mg protein)	0.24 ± 0.06^{a}	0.12 ± 0.02^{c}	0.22 ± 0.03^{ab}	0.13 ± 0.02^{bc}

C: Saline, AC: Acrylamide, IF: Intermittent fasting, ACIF: Acrylamide + intermittent fasting (n = 7 in each group). Different letters (a, b, c) indicate statistically significant difference. $P < 0.05$. Catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD)

Studies indicate that acrylamide induces oxidative stress in the kidneys. Rats administered AC at 38.27 mg/kg showed decreased levels of SOD and GSH and increased levels of malondialdehyde (MDA) in their kidneys [22]. AC also increases interleukin-1β which is an inflammatory interleukin [38].

In another study the gastric gavage administration of 50 mg/kg acrylamide to rats for 11 d leads to kidney damage. Different substances such as plant based ingredients [39] or endogenous molecules such as melatonin are tested to alleviate negative effects of AC [40]. However this current study presents another choice for mitigating adverse effects of AC via IF.

IF is known to exhibit protective effects against various conditions, including oxidative stress. IF is a process that supports the degradation of damaged organelles through autophagy and maintains cellular homeostasis. Important signaling molecules such as mechanistic Target of Rapamycin, Adenosine monophosphate-Activated Protein Kinase, and Sirtuin 1 are involved in this process. There is increasing evidence suggesting that IF plays a role in reducing damage in conditions of oxidative stress by restoring the oxidant-antioxidant balance and supporting autophagy [41]. IF enhances intracellular levels of the protective antioxidant GSH while reducing MDA, a terminal product of lipid peroxidation [42].

In diabetic rats, it has been shown that 4 weeks of IF reduces resistin, Sterol Regulatory Element-Binding Protein-1c (SREBP-1c) and inflammatory cytokines/enzymes (TNF-α, IL-6, IL-1ß, Myeloperoxidase (MPO)), and decreases the elevated levels of SOD, CAT, and GSH associated with diabetes [43]. This current study data demonstrate that IF reduces increased oxidative stress induced by acrylamide administration, aligning with literature findings. Notably, the scientific literature reports that alternating IF enhances antioxidant capacity and attenuates oxidative stress to a greater extent than restricted feeding [44], aligning well with our findings.

Histopathological findings

The livers of rats in the C and IF groups exhibited normal histological structure (FIG. 1a). In the AC group, moderate congestion and hepatocellular hydropic degeneration were present. Additionally, mild fatty degeneration, Kupffer cell proliferation, and bile duct proliferation were observed (FIG.1b and 1c). In the ACIF group, degenerative changes were mild (FIG.1d). Intermittent fasting was found to reduce the hydropic degeneration and congestion induced by AC in the liver (TABLE III).

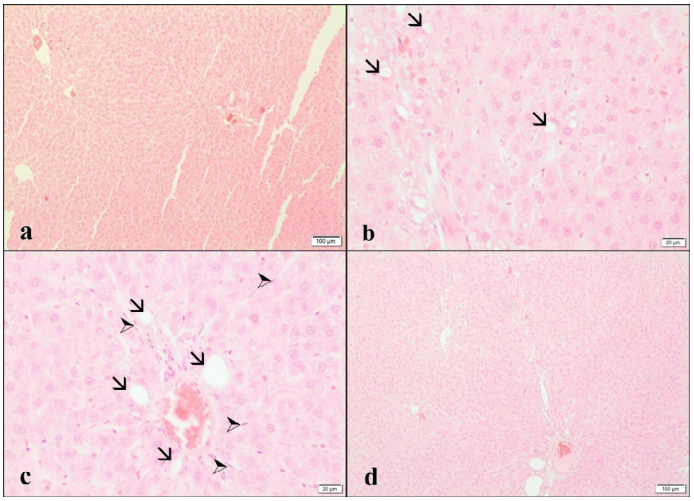

FIGURE 1. Histopathological findings observed in the liver, H.E. a) Normal histological structure in the liver, C group. b) Degenerative changes (arrows) in the liver, AC group. c) Bile duct proliferation (arrows) and Kupffer cell proliferation (arrowheads) in the liver, AC group. d) Mild degenerative changes in the liver, ACIF group. C: Saline, AC: Acrylamide, IF: intermittent fasting, ACIF: acrylamide+intermittent fasting (n = 7 in each group)

Significant hepatocellular hydropic degeneration was observed in the AC group. In the ACIF group, however, the presence of less damage and a similar pattern observed in the hyperemia parameter demonstrates a persistent detrimental effect of AC exposure. Bile duct proliferation was observed only in the AC group. Unlike these three parameters, fatty degeneration was observed in both the AC and ACIF groups.

In a study wistar rats were subjected to IF for 35 d including 18 h of fasting. Histopathological analysis revealed congested central vessels and dilated sinusoids, as well as increased Kupffer cell density, were less prominent in the IF groups and were found to resemble a normal hepatic appearance [45]. IF has been shown to have corrective effects on hepatotoxicity associated with diabetes (reducing blood sinusoids and inflammatory leukocyte infiltration) [46].

TABLE III
Liver histopathological findings

	C	AC	IF	ACIF
Hydropic degeneration in hepatocytes	-	++	-	+
Hyperemia	-	++	-	+
Bile duct proliferation	-	+	-	-
Fat degeneration	-	+	-	+

C: Saline, AC: Acrylamide, IF: intermittent fasting, ACIF: Acrylamide + intermittent fasting (n = 7 in each group).

Positive effects of fasting practices on liver and kidney function have also been reported in humans [47]. IF reduces inflammation, oxidative stress, and apoptosis in the senile rat liver, thereby alleviating histological deterioration [48]. The hepatoprotective effects of IF are also associated with several molecular mechanisms involving hepatic transcription factors (resistin, SREBP-1c), as well as IL-1β, IL-6, MPO and TNF-α [43].

In the kidneys, the C and IF groups exhibited a normal histological structure (FIG. 2a). Moderate tubular epithelial hydropic degeneration and congestion was present in the AC group (FIG. 2b and 2c). Mild degenerative changes were observed in the ACIF group (FIG. 2d) (TABLE IV).

In the kidneys, while tubular epithelial hydropic degeneration and congestion were observed in the AC group, only tubular epithelial hydropic degeneration was observed in the ACIF group, and this parameter was found to be milder compared to the AC group, indicating a protective effect on kidney tubular epithelial cells. Different studies in the literature have also shown histopathological damage caused by AC administration using various doses and administration protocols. Histopathological analyses of the kidneys in rats administered 38.27 mg/kg AC have revealed changes indicative of damage [22].

Conversely, IF decreases kidney injury during the progression from acute to chronic renal damage by attenuating fibrosis, inflammation, and oxidative stress [49]. IF has also been shown to reduce age-related renal inflammation, oxidative stress, apoptosis and to modulate autophagy activation in aged rats [50]. These histopathological results may be interpreted in light of the mechanisms outlined in the aforementioned studies.

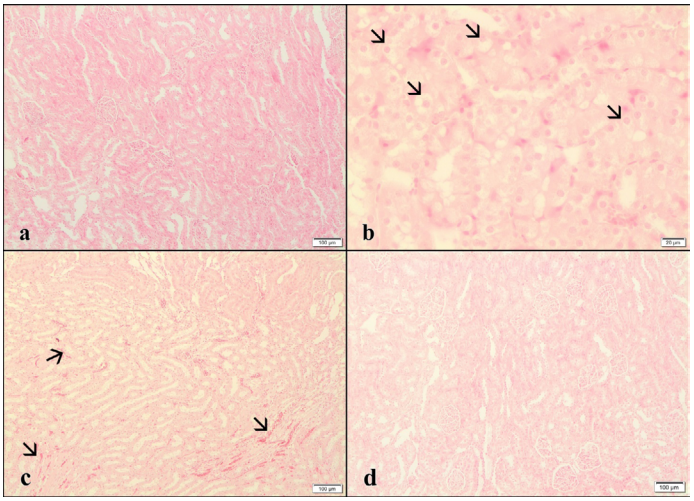

FIGURE 2. Histopathological changes observed in the kidneys. H.E. a) Normal histological structure in the kidneys, C group. b) Tubular epithelial cell hydropic degeneration in the kidneys, AC group. c) Congestion in the kidneys, AC group. d) Resembling normal structure in the kidneys, ACIF group. C: Saline, AC: Acrylamide, IF: intermittent fasting, ACIF: Acrylamide+intermittent fasting (n = 7 in each group)

TABLE IV
Kidney histopathological findings

	C	AC	IF	ACIF
Hydropic degeneration	-	++	-	+
Hyperemia	-	++	-	-

C: Saline, AC: Acrylamide, IF: intermittent fasting, ACIF: Acrylamide + intermittent fasting (n = 7 in each group).

CONCLUSION

Acrylamide exposure leads to significant effects in terms of histopathology, oxidative stress, behavior and serum biochemistry. Intermittent fasting cause a complete improvement in ACIF group in liver bile duct proliferation and kidney hyperemia as well as a partial improvement in CAT, SOD (kidney), CAT, GPX (brain) values, hydropic degeneration in hepatocytes and hyperemia in liver, hydropic degeneration in the kidney and rotarod walking period. As a result it can be concluded that intermittent fasting exerts a protective effect on rats in this experimental design.

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Conflict of interest

Authors declare no conflict of interest.

Ethical approval

All administrations were conducted in accordance with the Declaration of Helsinki with an ethical approval from Hacettepe university Animal Experiments Ethics Committee (decision number= 2020/11-09.

Author's contributions

All authors contributed to this project. First two authors contributed equally to this work.

BIBLIOGRAPHIC REFERENCES

[1] Arıhan O, Seringeç NB, Gürel EI, Dikmenoğlu NH. Effects of oral acrylamide intake on blood viscosity parameters in rats. Clin. Hemorheol. Microcirc. [Internet]. 2011; 47(1):45-52. doi: https://doi.org/qkrp

[2] Belai A, Burnstock G. Acrylamide-induced neuropathic changes in rat enteric nerves: similarities with effects of streptozotocin-diabetes. Auton Neurosci. [Internet]. 1996; 58(1-2):56-62. doi: https://doi.org/bjsf3n

[3] Pelucchi C, Galeone C, Levi F, Negri E, Franceschi S, Talamini R, Bosetti C, Giacosa A, La Vecchia C. Dietary acrylamide and human cancer. Int. J. Cancer. [Internet]. 2006; 118(2):467-471. doi: https://doi.org/fd3dtv

[4] Riboldi BP, Vinhas ÁM, Moreira JD. Risks of dietary acrylamide exposure: A systematic review. Food Chem. [Internet]. 2014; 157:310-322. doi: https://doi.org/gp4pmk

[5] Nowosad K, Sujka M. Effect of various types of intermittent fasting (IF) on weight loss and improvement of diabetic parameters in human. Curr. Nutr. Rep. [Internet]. 2021; 10:146-154. doi: https://doi.org/gpstdt

[6] Varady KA, Cienfuegos S, Ezpeleta M, Gabel K. Cardiometabolic benefits of intermittent fasting. Annu. Rev. Nutr. [Internet]. 2021; 41:333-361. doi: https://doi.org/gnzk9f

[7] Vitale R, Kim Y. The effects of intermittent fasting on glycemic control and body composition in adults with obesity and type 2 diabetes: a systematic review. Metab. Syndr. Relat. Disord. [Internet]. 2020; 18(10):450-461. doi: https://doi.org/gsvnk5

[8] Vasim I, Majeed CN, DeBoer MD. Intermittent fasting and metabolic health. Nutrients. [Internet]. 2022; 14(3):631. doi: https://doi.org/g9wmv7

[9] Francis N. Intermittent fasting and brain health: Efficacy and potential mechanisms of action. OBM Geriatrics. [Internet]. 2020; 4(2):121. doi: https://doi.org/qkrq

[10] Gudden J, Arias-Vasquez A, Bloemendaal M. The effects of intermittent fasting on brain and cognitive function. Nutrients. [Internet]. 2021; 13(9):3166. doi: https://doi.org/gpk4qr

[11] Neth BJ, Bauer BA, Benarroch EE, Savica R. The role of intermittent fasting in Parkinson's disease. Front. Neurol. [Internet]. 2021; 12:682184. doi: https://doi.org/gkbfxx

[12] Clifton KK, Ma CX, Fontana L, Peterson LL. Intermittent fasting in the prevention and treatment of cancer. CA Cancer J. Clin. [Internet]. 2021; 71(6):527-546. doi: https://doi.org/gpwf6s

[13] Berthelot E, Etchecopar-Etchart D, Thellier D, Lancon C, Boyer L, Fond G. Fasting interventions for stress, anxiety and depressive symptoms: A systematic review and meta-analysis. Nutrients. [Internet]. 2021; 13(11):3947. doi: https://doi.org/qkrr

[14] Fernandez-Rodriguez R, Martinez-Vizcaino V, Mesas AE, Notario-Pacheco B, Medrano M, Heilbronn LK. Does intermittent fasting impact mental disorders? A systematic review with meta-analysis. Crit. Rev. Food Sci. Nutr. [Internet]. 2023; 63(32):11169-11184. doi: https://doi.org/qkrs

[15] Tikoo K, Tripathi DN, Kabra DG, Sharma V, Gaikwad AB. Intermittent fasting prevents the progression of type I diabetic nephropathy in rats and changes the expression of Sir2 and p53. FEBS Lett. [Internet]. 2007; 581(5):1071-1078. doi: https://doi.org/fb65m3

[16] Xia E, Rao G, Van Remmen H, Heydari AR, Richardson A. Activities of antioxidant enzymes in various tissues of male Fischer 344 rats are altered by food restriction. J. Nutr. [Internet]. 1995; 125(2):195-201. doi: https://doi.org/pp6g

[17] Aebi H. Catalase *in vitro*. Methods Enzymol. [Internet] 1984;105:121-126. https://doi.org/dnf7v9

[18] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. [Internet]. 1967 [cited 10 Nov 2025]; 70(1):158-169. Available in: https://goo.su/FGBDzm

[19] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin. Chem. [Internet]. 1988; 34(3):497-500. doi: https://doi.org/gj74fn

[20] Abdel-Wahhab M, Nada S, Arbid M. Ochratoxicosis: prevention of developmental toxicity by L-methionine in rats. J. Appl. Toxicol. 1999; 19(1):7-12. doi: https://doi.org/drjc5b

[21] Şener G, Ekşioğlu-Demiralp E, Cetiner M, Ercan F, Şirvancı S, Gedik N, Yeğen BC. L-Carnitine ameliorates methotrexate-induced oxidative organ injury and inhibits leukocyte death. Cell. Biol. Toxicol. [Internet]. 2006; 22:47-60. doi:https://doi.org/cnsczn

[22] Gür F, Cengiz M, Gür B, Cengiz O, Sarıçiçek O, Ayhancı A. Therapeutic role of boron on acrylamide-induced nephrotoxicity, cardiotoxicity, neurotoxicity, and testicular toxicity in rats: Effects on Nrf2/Keap-1 signaling pathway and oxidative stress. J. Trace Elem. Med. Biol. [Internet]. 2023; 80:127274. doi: https://doi.org/qkrv

[23] Quan W, Li M, Jiao Y, Zeng M, He Z, Shen Q, Chen J. Effect of Dietary Exposure to Acrylamide on Diabetes-Associated Cognitive Dysfunction from the Perspectives of Oxidative Damage, Neuroinflammation, and Metabolic Disorders. J. Agric. Food Chem. 2022; 70(14):4445-4456. doi:https://doi.org/qkrw

[24] Kopańska M, Łagowska A, Kuduk B, Banaś-Ząbczyk A. Acrylamide Neurotoxicity as a Possible Factor Responsible for Inflammation in the Cholinergic Nervous System. Int. J. Mol. Sci. 2022; 23(4):2030. doi: https://doi.org/gp49sc

[25] Elias A, Padinjakara N, Lautenschlager NT. Effects of intermittent fasting on cognitive health and Alzheimer's

disease. Nutr. Rev. [Internet]. 2023; 81(9):1225-1233. doi: https://doi.org/qkrx

[26] Yang Q, Li M, Liu J, Zhang L, Yuan R, Xu Y, Zheng J, Cao S, Dai H, Liao M, Lv M, Chen X, Guo Y, Xie X, Zhang L, Chen X, Liang W. Intermittent fasting ameliorates neuronal ferroptosis and cognitive impairment in mice after traumatic brain injury. Nutrition. [Internet]. 2023; 109:111992. doi: https://doi.org/qkrz

[27] Chen W, Wang L, Zhou A, Fan C, Zhang Y, Wang Z, Rong S, Wang T. The benefits of intermittent fasting: A review of possible mechanisms on central neurological disorders. Acta Aliment. [Internet]. 2023; 52(1):1-11. doi: https://doi.org/qkr2

[28] Elesawy BH, Raafat BM, Muqbali AA, Abbas AM, Sakr HF. The Impact of Intermittent Fasting on Brain-Derived Neurotrophic Factor, Neurotrophin 3, and Rat Behavior in a Rat Model of Type 2 Diabetes Mellitus. Brain Sci. 2021; 11(2):242. doi:https://doi.org/qkr3

[29] Ebrahim HA, El-Gamal R, Sherif RN. Intermittent Fasting Attenuates High-Fat Diet-Induced Cerebellar Changes in Rats: Involvement of TNF-α, Autophagy, and Oxidative Stress. Cells Tissues Organs. 2021; 210(5-6):351-367. doi: https://doi.org/qkr4

[30] Ye Y, Chai SF, Li XR, Wu MN, Cai HY, Wang ZJ. Intermittent fasting and Alzheimer's disease—Targeting ketone bodies as a potential strategy for brain energy rescue. Metab. Brain Dis. 2023; 39(1):129-146. https://doi.org/qkr5

[31] Kaplan ML, Murphy SD. Effect of acrylamide on rotarod performance and sciatic nerve β-glucuronidase activity of rats. Toxicol. Appl. Pharmacol. [Internet]. 1972; 22(2):259-268. doi: https://doi.org/ddtrph

[32] Tanii H, Hashimoto K. Neurotoxicity of acrylamide and related compounds in rats. Effects on rotarod performance, morphology of nerves and neurotubulin. Arch. Toxicol. [Internet]. 1983; 54(3):203-213. doi: https://doi.org/dpb7wg

[33] Huang Z, Wang S, Yang Y, Lou J, Liu Z, Liu Z, Yong H, Shan S, Song F. Mitochondrial dysfunction promotes the necroptosis of Purkinje cells in the cerebellum of acrylamide-exposed rats. Food Chem. Toxicol. 2023; 171:113522. doi: https://doi.org/qksd

[34] Pak M, Bozkurt S, Pınarbaşı A, Öz Arslan D, Aksungar FB. Effects of Prolonged Intermittent Fasting Model on Energy Metabolism and Mitochondrial Functions in Neurons. Ann. Neurosci. 2022; 29(1):21-31. doi: https://doi.org/qksf

[35] Brocchi A, Rebelos E, Dardano A, Mantuano M, Daniele G. Effects of Intermittent Fasting on Brain Metabolism. Nutrients. [Internet]. 2022; 14(6):1275. doi: https://doi.org/qksg

[36] Ling B, Authier N, Balayssac D, Eschalier A, Coudore F. Assessment of nociception in acrylamide-induced neuropathy in rats. Pain. [Internet]. 2005; 119(1-3):104-112. doi: https://doi.org/dvbzsv

[37] Vanitha S, Thiagarajan VRK, Muthuraman A, Krishnan S, Aruna A, Tharabai R. Pharmacological evaluation of methanolic leaf extract of Swietenia mahagoni on acrylamide-induced neuropathic pain in rats. Toxicol. Ind. Health. [Internet]. 2015; 31(12):1185-1194. doi: https://doi.org/f3px9p

[38] Ajibare A, Akintoye O, Oriowo O, Asuku A, Adeola I, Ayoola A. Zinc Ameliorates Acrylamide-Induced Cognitive Impairment in Male Wistar Rats: Modulation of Oxidative Stress, Neuro-inflammation, and Neurotrophic Pathways. Biol. Trace Elem. Res. [Internet]. 2024; 203:4273-4282. doi: https://doi.org/qksh

[39] Cerrah S, Ozcicek F, Gundogdu B, Cicek B, Coban TA, Suleyman B, Altuner D, Bulut S, Suleyman H. Carvacrol prevents acrylamide-induced oxidative and inflammatory liver damage and dysfunction in rats. Front. Pharmacol. [Internet]. 2023; 14:1161448. doi: https://doi.org/qksj

[40] Ibaokurgil F, Aydin H, Yildirim S, Sengul E. Melatonin alleviates oxidative stress, inflammation, apoptosis, and DNA damage in acrylamide–induced nephrotoxicity in rats. Asian Pac. J. Trop. Biomed. [Internet]. 2023; 13(3):121-130. doi:https://doi.org/qksk

[41] Guo H, Bechtel-Walz W. The Interplay of Autophagy and Oxidative Stress in the Kidney: What Do We Know? Nephron. [Internet]. 2023; 147(10):627-642. doi: https://doi.org/qksm

[42] Abdel-Rahman M, Hussein AA, Ahmed-Farid OA, Sawi AA, Abdel-Moneim AE. Intermittent fasting alerts neurotransmitters and oxidant/antioxidant status in the brain of rats. Metab. Brain Dis. 2024; 39(7):1291-1305. doi: https://doi.org/qksn

[43] Agbonifo-Chijiokwu E, Nwangwa KE, Oyovwi MO, Ben-Azu B, Naiho AO, Emojevwe V, Ohwin EP, Ehiwarior AP, Ojugbeli ET, Nwabuoku SU, Moke EG, Oghenetega BO. Underlying biochemical effects of intermittent fasting, exercise and honey on streptozotocin-induced liver damage in rats. J. Diabetes Metab. Disord. [Internet]. 2023; 22(1):515-527. doi: https://doi.org/qksp

[44] Sharsher SI, Ahmed AI, Metwally M, Arisha AH, Ahmed KE. Intermittent Fasting Decreases Oxidative Stress Parameters and Increases Total Antioxidant Capacity. Biointerface Res. Appl. Chem. 2022; 12(5):6763–6775. doi: https://doi.org/qksq

[45] Ceylani T, Tekeli HT, Eranıl I, Yılmaz Ertük F, Keskin S. Intermittent Fasting Modulates Age-Dependent Morphological and Histological Hepatic Changes on Liver Tissue in Wistar Rats. In: Oral O, Nalci KA, editor. New Frontıers In Health Scıences. Türkiye: Duvar Yayınları; 2023; 8:125-142. Available in:https://goo.su/16Y6Gq

[46] Al-Kazimi N, Jarrar Y, Abdul-Wahab G, Alsayed AR, Madani A, Abulebdah D, Musleh RS, jarrar Q, Al-Ameer HJ, Al-Awaida W, Abdullah E. Effects of intermittent fasting on the histology and mRNA expression of major drug-metabolizing cyp450s in the liver of diabetic mice. Libyan J. Med. [Internet]. 2023; 18(1):2270188. doi: https://doi.org/qkss

[47] Priyono D, Harun H, Viotra D, Dasril ZM. Effect of Ramadan Fasting on DNA Repair, Immune System, Inflammation and Cognitive Function in Chronic Kidney Disease Patients: A Systematic Literature Review. Bio. Sci. Med. J. Biomed. Transl. Res. [Internet]. 2023; 7(4):3252-3256. doi: https://doi.org/qkst

[48] Hafez SMNA, Elbassuoni E. Dysfunction of aged liver of male albino rats and the effect of intermitted fasting; Biochemical, histological, and immunohistochemical study. Int. Immunopharmacol. 2022; 103:108465. doi: https://doi.org/qksv

[49] Jang Y, Kim YS, Kim SR, Lee DW, Lee SB, Kim IY. Intermittent Fasting Protects Against the Progression from Acute Kidney Injury to Chronic Kidney Disease. Antioxidants. 2025; 14(1):119. doi: https://doi.org/g89gtf

[50] Rifaai RA, El-Tahawy NFG, Abozaid SMM, Abdelwahab A. Intermittent Fasting Ameliorates Age-Induced Morphological Changes in Aged Albino Rat Kidney via Autophagy Activation and Reduction of Apoptosis and Inflammation. Microsc. Microanal. 2025; 31(1):ozae102. doi: https://doi.org/qksw