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Fischer's ratio and DNA damage in hypoxemia-induced brain injury in rat model: prophylactic role of quercetin and mexamine supplementation

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Abstract

Hypoxemia brain injuries arise when the brain's oxygen supply is restricted. Brain cells gradually die and become impaired as a result of the restricted oxygen flow a diversity of signaling pathways are involved in the pathophysiology of brain damage. One of the main concerns when examining the rate of protein breakdown is the measurement of the serum amino acid ratio. Valine, leucine, and isoleucine make up branched-chain amino acids, while phenylalanine and tyrosine make up aromatic amino acids. A vital tool for assessing the severity of hypoxemia is Fischer's ratio. The goal of this article is to determine how quercetin (QUR) and/or mexamine (MEX) prevented synfat (SN)-induced brain damage in a rat models. It also aimed to elucidate the various cross-linked inflammatory pathways, DNA damage, and Fischer's ratio. Following QUR and MEX therapy, synfat-induced hypoxemia. Hemoglobin (Hb) levels were markedly reduced by synfat-intoxication, and oxidative stress and inflammatory biomarkers, including TNF-??, MDA, interleukin-6 (IL-6), and C -reactive protein (CRP), were elevated. Hemoglobin levels, oxidative stress biomarkers, and the aberrant expression of pro-inflammatory cytokines were all altered by QUR and/or MEX therapy. Similarly, the concentration of γ-aminobutyric acid, serotonine, noradrenaline, and intropin in cerebral tissue is restricted. Similarly, the COMET assay and 8-oxo-7,8-dihydro-2'-deoxyguanosine analysis (8-oxodG) demonstrated that QUR and MEX potentially altered synfat-induced brain DNA damage. The results confirmed the potential impact of this combined strategy as a powerful therapy for brain hypoxemia, concluding that treatment via QUR with MEX was superior therapy in modulating synfat-triggered cerebral injury.

Introduction

The brain's ability to function is disrupted instantly if the oxygen supply is cut off, and irreparable harm may ensue shortly after. We call this anoxic or hypoxic brain injury. For the brain to survive, oxygen must be continuously supplied. The brain's primary energy source, glucose, cannot be utilized without oxygen. If the oxygen supply is cut off, consciousness will be lost in 20 seconds, and after 5 minutes or so, brain damage will start to happen.

Cerebral anoxia is the term used to describe a total disruption of the oxygen flow to the brain. Cerebral hypoxemia occurs when there is still a partial supply of oxygen, but not enough to sustain regular brain function. These two terms are frequently used interchangeably in practice. According to the Glasgow Coma Scale (GCS), there is some evidence that severe anoxic brain injury may have a worse prognosis than a traumatic brain injury. This could potentially be due to variations in the type of brain damage. Damage to the axons is typically a noticeable aspect of traumatic brain injury, and the brain can frequently form new axons to make up for this. The actual nerve cell bodies themselves are extremely susceptible to damage in anoxic brain injury, and this damage is irreversible.

The effects of hypoxemia on neuronal tissue are exacerbated by the release of multiple pro-inflammatory mediators from neurons and glia. It is recognized that during the initial phases of hypoxemia, interleukin-1β, and TNF-α cytokines are released, causing local and systemic inflammation as well as additional DNA damage and cell death. The pathophysiology of several cerebral-related syndromes depends heavily on oxygen homeostasis, and disruption of this balance is crucial for many physiological developments [1]. A harmful condition known as hypokinetic hypoxemia results from deficient blood flow, which lowers oxygen supply to tissues. Synfat ($NaNO_2$; SN) is an inorganic salt utilized in chemical manufacturing, coloring agents, and meat treatments. Because of its strong affinity for hemoglobin (Hb), SN reduces the protein's ability to bind oxygen, resulting in methemoglobinemia. It also generates nitrogen dioxide (NO_2) and hydrogen peroxide (H_2O_2), which both contribute to hypoxia [2]. On the other hand, nitrogen dioxide oxidizes ferrous-Hb to met-Hb, and hydrogen peroxide oxidizes methemoglobin to ferryl-Hb radical. Nitrosative stress, which is caused by reactive nitrogen species, is widely recognized as a major mediator of cellular structural damage, affecting proteins, lipids, membranes, and DNA. Hypoxemia increases the level of lipid peroxidation and decreases the activity of brain antioxidants [3]. The electrochemical impulse transfer between brain cells and the maintenance of neuronal function depends heavily on oxygen. Within a few minutes, brain hypoxia causes cell death, which impairs brain function and produces free radicals [4]. Stress-related degenerative stressors like ROS, superoxide anion radical, H_2O_2, and OH radical can cause severe damage to the brain. Lipid peroxidation in brain membranes is brought on by these stressors. The metabolism of lipids, proteins, carbohydrates, and electrolytes is impacted by hypoxia [5]. Brain injury results from the administration of synfat because it causes oxidative stress, inflammation, ischemia, hypoxia, and decreased metabolic energy [6,7]. The digestive, respiratory, neurological, endocrine, and urinary systems are all harmed by hypoxemia. Furthermore, oxidative stress is a mediator of brain DNA damage [8]. TNF-?? and IL-6 are two examples of inflammatory cascades that play a crucial role in inflammation and increase CRP synthesis [9].

Memory, mood, motor coordination, learning, and many other physiological, pharmacological, and behavioral processes are influenced by the biogenic amines, adrenaline, noradrenaline, and dopamine [10]. Hypoxia is a neurodegenerative disease that lowers brain levels and causes behavioral, neurochemical, and other problems [11]. A monoamine neurotransmitter called serotonin stimulates neuromodulation of the respiratory rhythm [12]. Reduced ventilatory response to hypoxemia is associated with Prader-Willi disorder, which is characterized by disruption of serotonine metabolites in the cerebrospinal fluid [13].

Originating from L-glutamate decarboxylation, γ-aminobutyric acid (GABA) plays a vital role in blood pressure regulation, sympathetic nervous system activity, and endocrine system function [14]. In rats, hypoxia leads to an overabundance of glutamate, which exacerbates seizures and damages neurons [15]

. Metabolism of Branched-chain amino acids (BCAAs), that is composed of leucine, valine, and isoleucine, occurs in the muscle [16], whereas, aromatic amino acids (AAAs), including tyrosine and phenylalanine, are broken down in the hepatocytes [17]. Fischer's ratio [BCAAs/AAAs concentrations] is vital for categorizing the hypoxemia intensity [18].

An indole analog that penetrates the blood-brain barrier and builds up in the cerebellum is mexamine (N-acetyl-5-methoxytryptamine; MLN) [19]. Through the activation of NF-κB and the inhibition of transcription factors, it has the capability to scavenge ROS and play an anti-inflammatory role [20], transcript inflammatory cytokines such as CRP, interleukin-6, and tumor necrosis factor-α, and preserve DNA mutations [21]. Many fruits, drinks, and vegetables contain quercetin, which has anti-oxidant, antiproliferative, and anti-inflammatory qualities [22]. In a number of injured models, QRC protects the brain and lungs [23].

The current study uses biochemical measurements of inflammatory biomarkers, neurotransmitter levels, and DNA fragmentation to demonstrate the preventive potential of MEX and/or QUR against synfat-induced cerebral injury.

Materials and methods

Chemicals

The manufacturers of MEX Cat # 73-31-4 and Querectin Cat #117-39-5 were Sigma-Aldrich Co. (St. Louis, MO, USA). The Randox Company provided the kits used for hemoglobin determination (UK). Every chemical was of the highest analytical quality.

Experimental animals

Wistar male albino rats weighing (NO.#40,180–190 g), aged 12 weeks, were obtained from the National Research Centre Animal House in Egypt. The procedures outlined by the NRC's Experimental Animal Ethics Committee (34512012023) were monitored and approved. The rats were kept in standard humidity and temperature settings. They had unlimited access to tap water and were given regular rat pellet chow. Eight rats were split up into five groups:

G 1: control group was treated with normal saline.

G 2: Synfat- treated rats with doses of (60 mg/kg) divided over six days subcutaneously.

G 3: Synfat- rats treated with QUR (200 mg/kg, i.p.) 24 h after synfat injection for one month [19].

G 4: Synfat - rats treated with MEX (200 mg/kg, i.p.) 24 h post synfat injection for one month [20].

G 5: Synfat - rats treated intraperitoneally with both QUR (200 mg/kg) and MEX (200 mg/kg) for one month.

Synfat, MEX, and QUR were emulsified in one drop of carboxy methyl cellulose and then dissolved in normal saline.

Following SN administration and treatment, blood was sampled and cerebral tissue was separated; isoflurane (3.8 ± 1.1 min) was used as an anesthetizer. After that, rats were sacrificed and beheaded. Blood was extracted and divided into two portions. The first portion was placed in tubes containing EDTA for Hb analysis, and the second portion was centrifuged to create serum and kept at -70 °C for additional examination. Rats' brain tissues were separated and homogenized in PBS to create a 20% homogenate.

Estimation of Hemoglobin (Hb)

Drabkin's reagent was used to determine IHb [24]. Briefly, 20 μL of blood was combined with 5 mL of Drabkin's solution. After 15 minutes of room temperature incubation, the mixture's color absorbance at 540 nm was measured using a spectrophotometer (V-760 UV-visible) in

comparison to the blank. This is predicated on hemoglobin oxidizing with potassium ferricyanide to produce methemoglobin, which subsequently reacts with potassium cyanide to form cyanomethemoglobin. Its maximum absorption occurs at 540 nm.

Biochemical serum investigation

The ELISA kit (IBL-GmbH, Germany) was used to quantify interleukin-6. The ELISA assay kit (R&D Systems-Minneapolis, MN, USA) was used to measure tumor necrosis factor-α. [25]. Using a Behring Nephelometer-II analyzer, immunome phelometric analysis of C-reactive protein (Dade Behring N Latex High Sensitivity-CRPTM mono-assay) was performed.

γ-aminobutyric acid, noradrenaline, serotonine, 8-oxodG and Intropin analysis

Serotonine (Cat # MBS225497), Intropin (Cat # MBS525908), GABA, and 8-oxodG (Cat # MBS025103) concentrations in brain tissues were quantitatively ascertained using ELISA kits, in accordance with the manufacturer's protocol (R&E Co., NADR, Cat # MBS033557). The homogenate of noradrenaline, GABA, and 8-oxodG was made 1:10 PBS, while the homogenate of intropin and serotonine was made 1:1 w/v (homogenization of cerebral tissue in PBS). Next, the homogenates were centrifuged for 15 minutes at 4000 rpm.

This procedure is based on a competitive enzyme immunoassay technique that uses noradrenaline/Intropin/GABA HRP serotonine conjugates and polyclonal anti-SER/NADR/Intropin/GABA/8-oxodG antibodies. 100 μL of brain sample, BPS (as), or standard is used to create the blank. It is then incubated with 50 μL of serotonine/noradrenaline/intropin/GABA/eight-oxodG HRP conjugates (in a pre-coated plate) for one hour at room temperature. After that, the wells were incubated, decanted, and washed five times. After that, the HRP-enzyme substrate was added to the wells, then the plate was kept in the dark until a blue hue developed. Finally, the stop solution was changed. Color intensity (450 nm) is measured using a spectrophotometer [26,27].

Comet assay

A straightforward method for quantitative assessment of DNA strand breakage is the comet assay, which is known as single-cell gel electrophoresis, also. In order to create supercoiled DNA loops connected to the nuclear matrix, cells were run on agarose and lysed with detergent to create nucleoids. Comet-like structures produced by electrophoresis are visible under fluorescence microscopy; the quantity of DNA breaks is indicated by the intensity of the COMET tail in relation to the head. Breaking loops are able to migrate freely to the anode and miss supercoiling [28].

Estimation of amino acid concentrations

Concentrations of cerebral amino acids were examined by HPLC assay [29].

Calculation of Fischer's ratio

Fischer's ratio was calculated by dividing BCAAs/AAAs levels [30].

Statistical analysis

Results were demonstrated as means ± SEM. The statistics were accomplished using a one-way analysis of variance (ANOVA) proceeded by the Tukey-Kramer multiple comparisons test. Significance was set at $p \leq 0.05$. Statistical tests were performed via SPSS 16. Graphs were performed with GraphPad prism 10 program.

Results

Potential of QUR and MEX on Hb level

With a mean value of 6.1 correlated with the normal value, Synfat explained a significant drop in Hb-concentration. The Hb level was significantly altered by QUR, MEX, and their combination; the combination regimen showed the biggest effect, with a mean value of 10.9 ($P < 0.05$), Table 1.

Potential of QUR and MEX on inflammatory biomarkers

Synfat rats' brain CRP, IL-6, TNF-α, and IL-6 levels were significantly higher than control values, with mean values of 18.2, 65, and 420, respectively. When compared to synfat rats, QUR and MEX therapy significantly decreased the triggered inflammatory cytokines; the combination regimen showed the greatest potential, with mean values of 13, 45, and 299, respectively ($P < 0.05$) (Fig. 1).

Potential of QUR and MEX on monoamine neurotransmitters

The neurotransmitters intropin, GABA, serotonine, and noradrenaline all showed a discernible decrease in response to hypoxemia, with mean values of 13.5, 2.1, 4.8, and 3.1, respectively (Fig.2). However, compared to synfat rats, QUR or MEX therapy altered the improvement in noradrenaline, intropin, serotonine, and GABA levels. The most successful combination, however, was QUR and MEX, with mean values of 17.1, 5, 6.14, and 4.9, respectively.

Potential of QUR and MEX on brain DNA damage

In comparison to the control value, the synat-hypoxemic group showed a noticeable increase in tail moment, length, and DNA percent, indicating remarkable DNA damage. DNA damage in relation to the synfat group was lessened by QUR and MEX therapy. The modulatory effects of QUR and MEX on cerebral DNA damage following elevation in the synfat group are depicted in Figs 3 and 4. While mexamine and the combination regimen indicated low DNA damage, QUR displayed moderate DNA damage. Because damaged DNA migrates to the tail, the length of the tail increases as the DNA damage percentage increases.

Potential of QUR and MEX on 8-oxodG

8-oxodG level in synfat hypoxemic rats was markedly increased by a mean value of 85.9 pg/ml, than that of the control value of 26.2 pg/ml. Nevertheless, QUR and MEX therapy markedly reduced the triggered oxidative-DNA damage biomarker, compared with synfat rats with the combination regimen revealing the highest power with a mean value of 32.5 pg/ml ($P < 0.05$) (Fig. 5).

Table 1. Effect of QUR and/or MEX on hemoglobin (Hb) level in hypoxemic rats.

Group	Hb
Control	11.95 ± 1.6
Synfat	5.1 ± 2.6*
Synfat & QUR	10.0 ± 3.8$
Synfat& MEX	9.1 ± 2.27$
Synfat & QUR & MEX	10.96 ± 3.6$

Data are expressed as mean ± SD; n = 10. $P \leq 0.05$ *: Significantly different from the control group. $: Significantly different from the synfat-treated animals.

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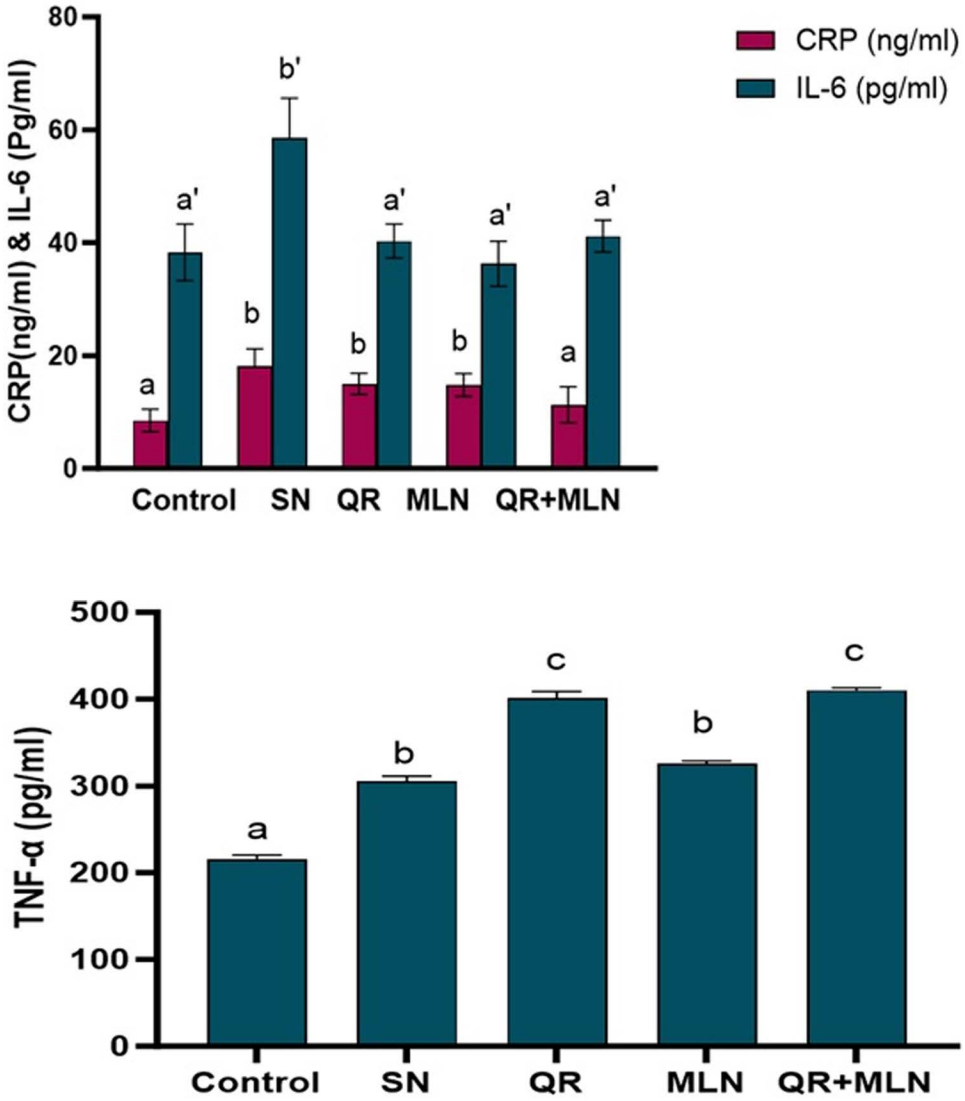

Fig 1. A&B Impact of QUR and MEX on serum CRP, IL6, and TNF- α levels in the control and hypoxemia-group. Data are presented as mean ± S.E.M (n = 8). **p** ≤ 0.05 value is considered significant. Groups carrying the same letter are not significantly different from each other, while those having different letters are significantly different from each other using ANOVA, followed by Bonferroni as a post-ANOVA test.

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Potential of QUR and MEX on malondialdehyde

MDA level in synfat hypoxemic rats was noticeably augmented by a mean value of 69.2 nmol/gm tissue, than the control value. Nevertheless, QUR and MEX therapy obviously ameliorated the triggered oxidative stress biomarker MDA, compared with synfat rats with the combination regimen enlightening the uppermost impact with a mean value of 15.7 nmol/gm tissue ($P < 0.05$) (Fig. 6).

Histopathological investigation

Control group represented normal nerve fibres, neuronal cells and neuroglia cells. Hypoxemia group revealed noticeable degeneration in neuronal cells while neuropil does not change.

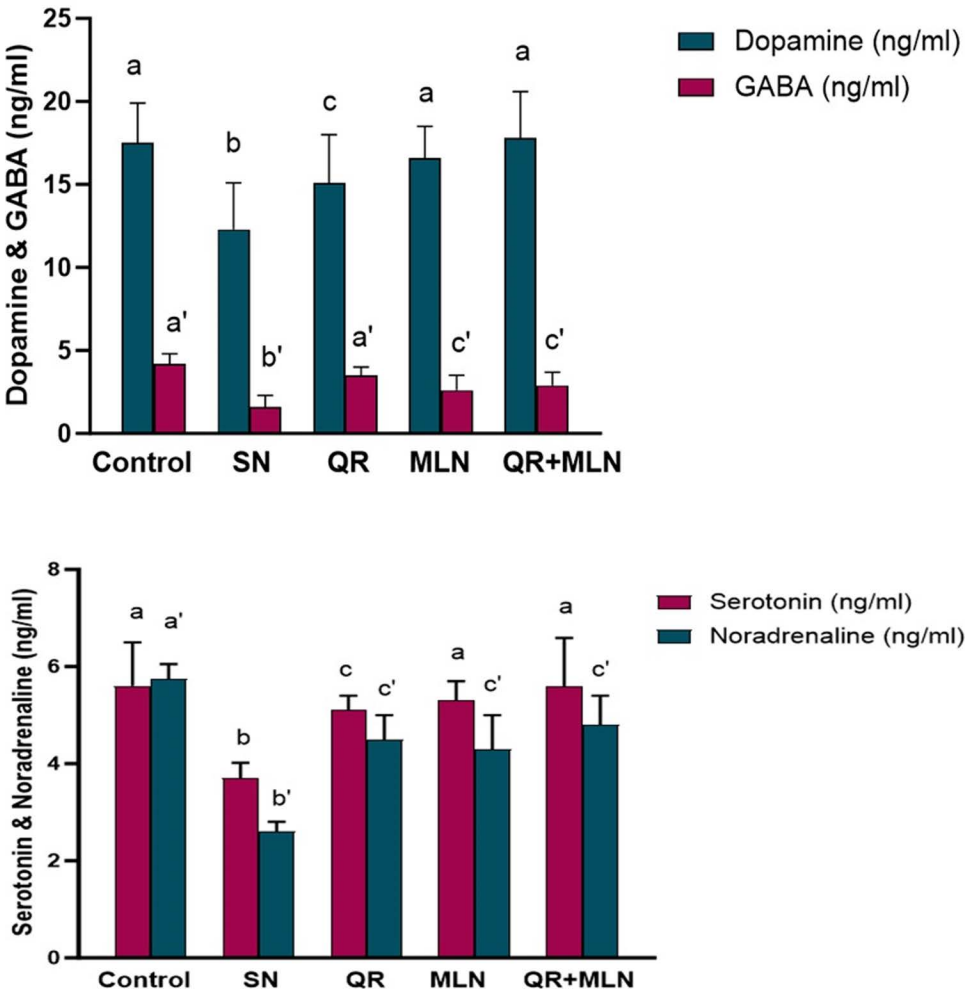

Fig 2. Impact of QUR and MEX on brain tissue Intropin, GABA, serotonine, and noradrenaline levels in the control and hypoxemia- groups. Data are expressed as mean ± S.E.M (n = 8). **p** ≤ 0.05 value is considered significant. Groups having the same letter are not significantly different from each other, while those having different letters are significantly different from each other using ANOVA, followed by Bonferroni as a post-ANOVA test.

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MEX therapy, revealed a few degenerated nerve fibers, neuronal cells and neuroglia cells. QUR therapy revealed moderately hypertrophied neuronal cells. Hypoxemic rats received both QUR and MEX therapy, which revealed normal neuronal cells, perfect nerve fibers and neuroglia cells (Fig.7).

Potential of QUR and MEX on the level of cerebral amino acids (nmol/ml) and Fisher's ratio

In contrast to the non-intoxicated animals, synfat-intoxication produced a marked increase in AAA, tyrosine, phenylalanine, alanine, tryptophan, isoleucine, and taurine levels, as well as an extremely significant alteration in Fisher's ratio, accompanied by levels of histidine, proline, and citrulline (Tables 2 and Fig 8). A heatmap relating various amino acids is shown in Fig 8.

While the administration of QUR and MEX decreased tyrosine and phenylalanine and tyrosine levels in comparison to synfat rats, the administration of QUR and MEX

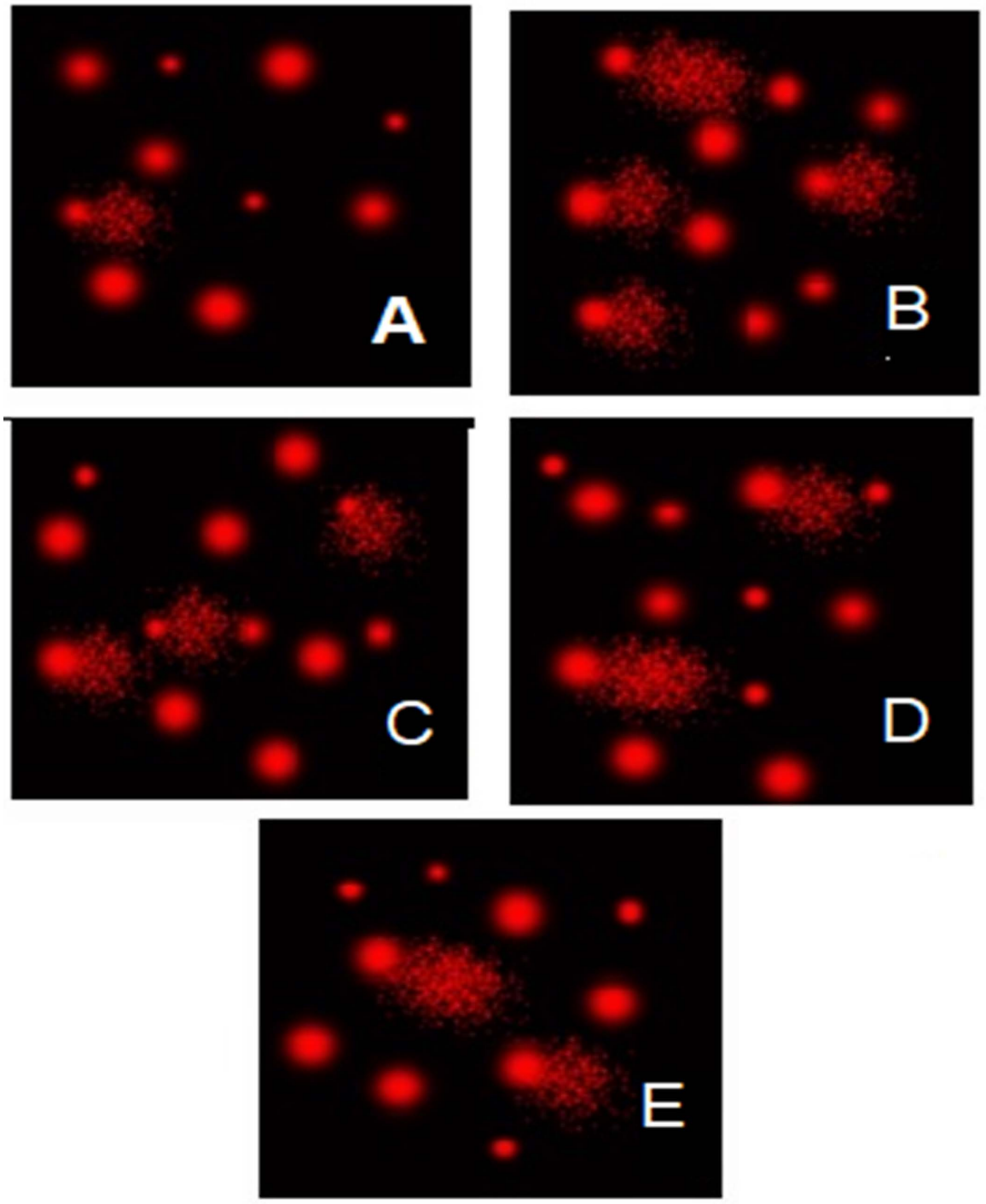

Fig 3. DNA damage in the brain tissues of synfat- hypoxemic rats and the impact of QUR and MEX treatment on the level of DNA damage. Comet assay showing the degree of DNA damage in the brain tissues of (A) normal control group, (B) group intoxicated with the synfat, (C) intoxicated group treated with QUR, (D) intoxicated group treated with MEX (E) intoxicated group treated with QUR and MEX.

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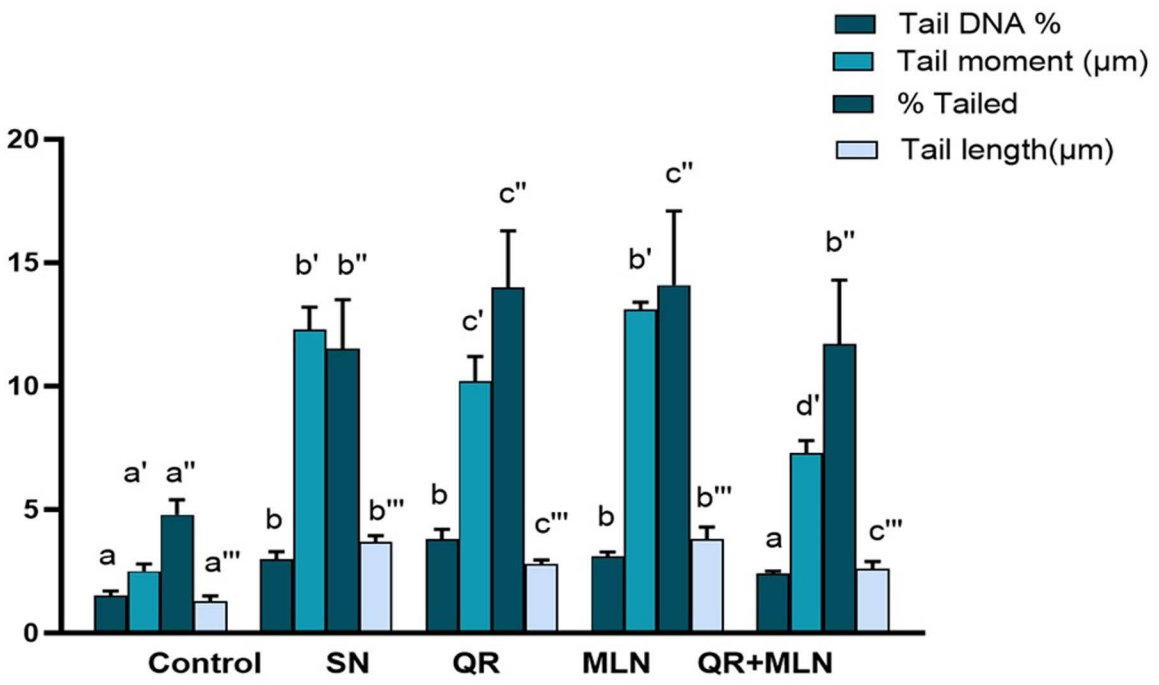

Fig 4. Impact of QUR and MEX on brain DNA-damage in the control, hypoxemic and treated groups. Data are presented as mean ± S.E.M (n = 8). **p** ≤ 0.05 value is considered significant. Groups carrying the same letter are not significantly different from each other, while those having different letters are significantly different from each other using ANOVA, followed by Bonferroni as a post-ANOVA test.

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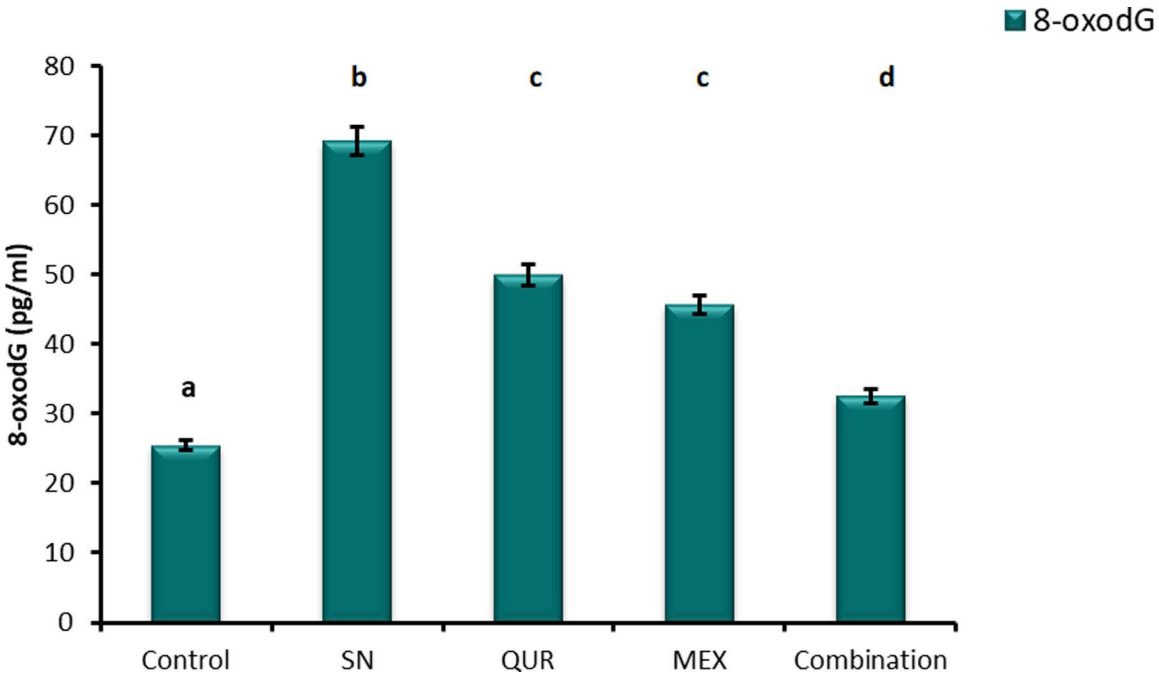

Fig 5. Impact of QUR and MEX on brain oxidative DNA- damage biomarker 8-oxodG in the control, hypoxemic and treated groups. Data are presented as mean ± S.E.M (n = 8). **p** ≤ 0.05 value is considered significant. Groups carrying the same letter are not significantly different from each other, while those having different letters are significantly different from each other using ANOVA, followed by Bonferroni as a post-ANOVA test.

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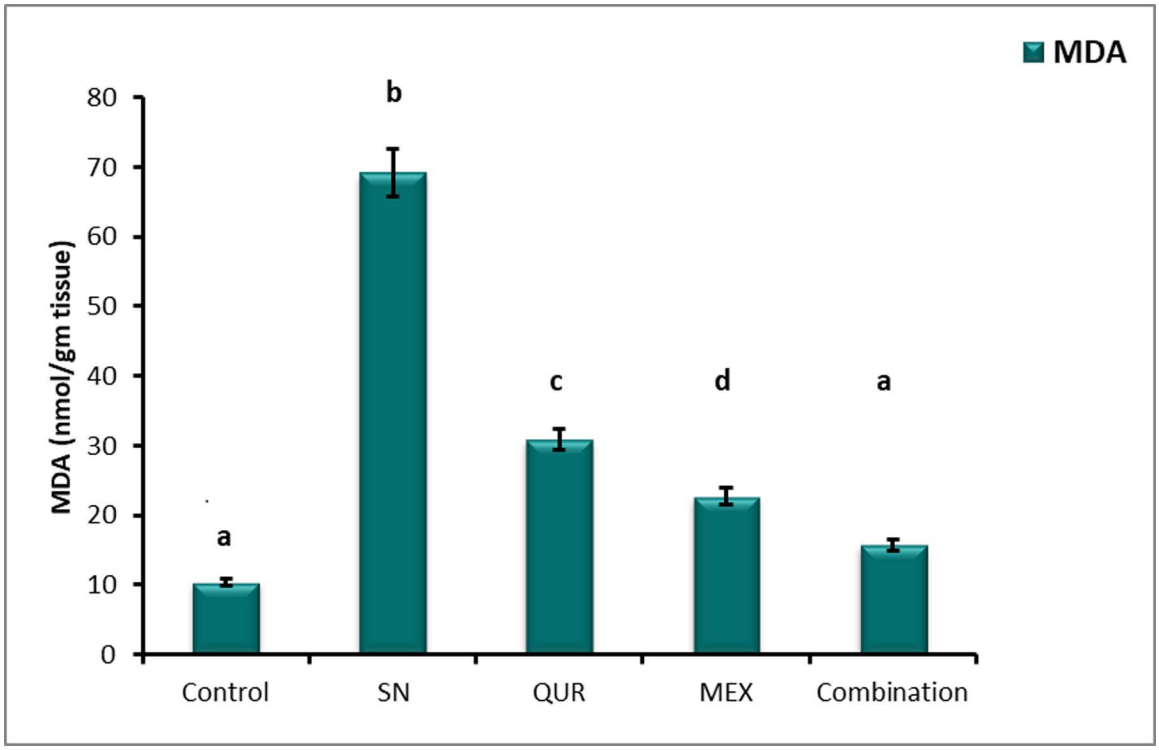

Fig 6. Impact of QUR and MEX on brain oxidative stress biomarker malondialdehyde in the control, hypoxemic and treated groups. Data are presented as mean ± S.E.M (n = 8). **p** ≤ 0.05 value is considered significant. Groups carrying the same letter are not significantly different from each other, while those having different letters are significantly different from each other using ANOVA, followed by Bonferroni as a post-ANOVA test.

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and their combination caused a significant decrease in AAA levels. When compared to the normal, the synfat group's NH3 and urea levels were noticeably higher. Interestingly, the significant drop in urea level confirmed the combined effect of QUR and MEX on synfat rats.

The concentrations of total AAA and total branched-chain amino acids (BCAA) decreased considerably in the groups treated with QRC and/or MLN. in contrast to the hypoxic cohort. Fisher's ratio was significantly higher after MEX or in combination with QUR than it was in the synfat group, but it was significantly lower in treated groups with QUR than in the normal value.

Discussion

Hypoxemic lack of oxygen is the main etiology of cerebral injury, it can contribute to cerebral hypoxia. Confusion, trouble speaking, and seizures are among the main symptoms. It is a potentially lethal medical emergency that can result in permanent brain damage. Medical professionals can address some of the problems caused by cerebral hypoxia, but they are unable to undo the potential harm to the brain thus early intervention is a must. All bodily metabolic processes are disrupted by hypoxemia, which results in a lack of energy for the brain and ultimately leads to death [31]. Nitrites are toxic through two mechanisms: cardiovascular influence and methemoglobinemia, which is characterized by hypoxemic tissues and results

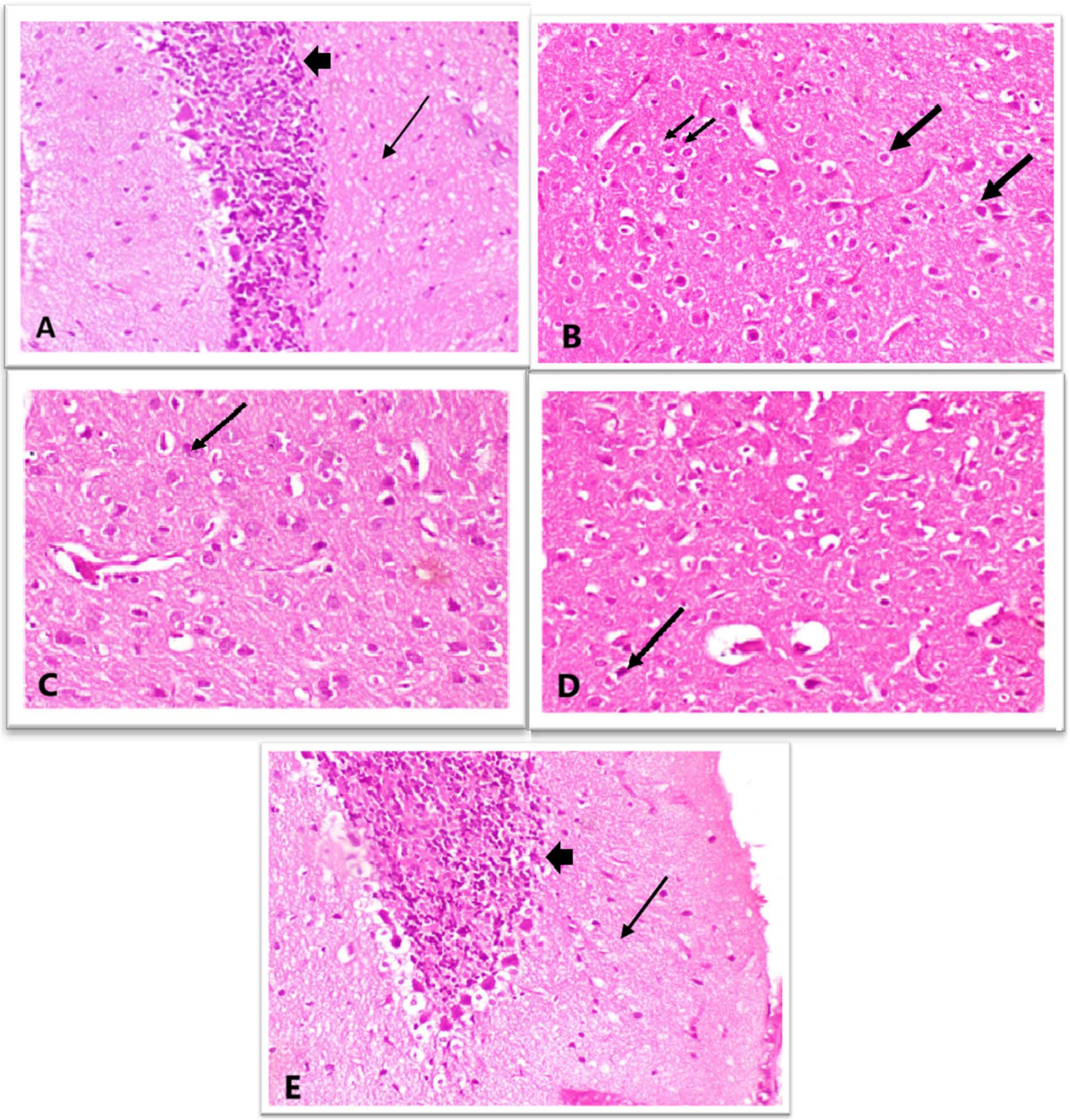

Fig 7. Histopathological investigation and light photomicrographs of rat's brain tissue stained with Hematoxylin and Eosin (Scale bar: 50μm), in which (A) **Represent normal neurons (long arrow) and nerve fibres and glial cells (short arrow).** (B) Section of brain exposed to hypoxemia representing marked deterioration of neuronal cells (arrow head) while neuropil does not change (arrow). (C) Hypoxemic rats received QUR treatment showing moderately hypertrophied neurons (D) Hypoxemic rats received MEX treatment, revealing few degenerated neurons, nerve fibres and neuroglia cells (E) Hypoxemic rats received both QUR and MEX treatments, showing normal neurons perfect nerve fibres and neuroglia cells.

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Table 2. Cerebral amino acid and different nitrogenous compounds concentrations in (nmol/ml) as well as Fischer's ratio in hypoxic rats, Mexamine and Querectin treated groups.

	Control	Synfat	Querectin	Mexamine	QR & MEX
Aspartic acid	30.3 ± 8.6	25.3 ± 3.8*	27.5 ± 3.1	27.9 ± 8.6	29.1 ± 4.7
Hydroxyproline	48.5 ± 7.1	32.4 ± 3.2*	40.1 ± 4.1	38.4 ± 6.8	44.9 ± 6.5
Threonine	295.3 ± 45.1	257.2 ± 12.3***	282.3 ± 10.4***, $$$	285.7 ± 20.1***, $$$	288.4 ± 21.6***, $$$
Serine	185.4 ± 9.8	215.7 ± 10.4**	171.3 ± 13.4$$$$	193 ± 20.5	191 ± 9.3
Asparagine	50.5 ± 5.2	41.8 ± 4.1**	46.5 ± 4.9	41.4 ± 9.2**	48.9 ± 4.6$$
Glutamine	808.7 ± 14.1	840.4 ± 14.2***	819 ± 8.6$$	800.8 ± 4.7$$$	508 ± 7.9***,$$$
Proline	148.33 ± 2.3	118.9 ± 9.8***	138.5 ± 10.2	130.9 ± 9.8**	139.6 ± 10.4$$
Glycine	217.5 ± 8.8	194.8 ± 4.6	200.5 ± 5.8	188.3 ± 10.9	206.5 ± 12.5
Alanine	401.9 ± 20.9	490.1 ± 5.8***	429.9 ± 7.4**	437.9 ± 10.6**	421.9 ± 5.8
Citrulline	103.8 ± 9.6	68.9 ± 5.5***	88.4 ± 6.2***,$$$	82.5 ± 3***,$$$	99.7 ± 2.5$$$
Valine	201.9 ± 13.4	232.5 ± 11.6***	217.9 ± 15.0***,$$$	223.1 ± 10.9***,$$$	212.9 ± 9.1 ***,$$$
Methionine	55.9 ± 5.6	65.9 ± 4.8**	56.8 ± 3.85	61.9 ± 2.8$$	61.1 ± 3.5
Isoleucine	98.1 ± 6.4	144.9 ± 5.6	95.9 ± 3.5	141.13 ± 4.4	131.1 ± 10.2
Leucine	188.1 ± 9.2	225 ± 10.5***	209.9 ± 20.2$$$	191.9 ± 0.65***,$$$	184 ± 6.9***,$$$
Tyrosine	58.8 ± 4.3	145.8 ± 2.9***,$$$	100.3 ± 3.7***,$$$	98.1 ± 5.9***,$$$	100 ± 4.3***,$$$
Phenylalanine	77.3 ± 7.8	163.5 ± 5.8***	89.8 ± 6.3*,$$$	94.7 ± 5.8*,$$$	89 ± 5.4$$$
Histidine	73.5 ± 2.1	62.2 ± 1.1**	57.1 ± 2.9***	59.8 ± 3.4**	61.4 ± 2.4*
Tryptophan	101.6 ± 10.3	116.1 ± 9.3*	102.9 ± 2.5	110.2 ± 6.2	107 ± 4.2
Ornithine	77.4 ± 4.1	89.9 ± 2.4*	77.1 ± 2.8$	76.9 ± 1.1$	80 ± 4.8$
Lysine	338.9 ± 2.7	353.7 ± 1.6**	319.8 ± 2.2***,$$$	334.9 ± 9.9$$$	341.8 ± 5.6$$
Arginine	312.2 ± 10.6	285 ± 6.1	300.9 ± 9.8	290.1 ± 17.9	307 ± 9.5
α-aminoadipic acid	31.7 ± 1.5	15.9 ± 2.3***	22 ± 2.7**	23.1 ± 1.5*	28.6 ± 1.5$$
1-methylhistidine	2 ± 0.1	2 ± 0.17	1.75 ± 0.4	1.44 ± 0.1	1.59 ± 0.31
Urea	3229 ± 10.1	7333 ± 19.2***,$$$	6418 ± 20.1***,$$$	6511 ± 31.3***,$$$	6303 ± 10.8***,$$$
Asparagine	295 ± 5.1	298.2 ± 7.6	289.2 ± 8.7**,$$$	272.2 ± 20.6***,$$$	276.8 ± 15.1$$$
Ammonia	188 ± 6.3	321 ± 5.1***	185 ± 4.4$$$	192 ± 9.8$$$	189 ± 5.8$$$
Aminobutyric acid	9.01 ± 3.8	6.1 ± 1. 4	7.9 ± 2.6	6.4 ± 1.1	7.9 ± 1.8
Taurine	140 ± 7.4	228 ± 21.5**	190 ± 18.5	202 ± 13.5*	212 ± 24.4*
Hyomocystine	0.7 ± 0.12	0.819 ± 0.11	0.615 ± 0.05	0.7 ± 0.02	0.69 ± 0.03
BCAA	489.1 ± 4.6	603.4 ± 14.7***	525.2 ± 15.5***,$$$	554 ± 10.5***,$$$	528.03 ± 6.9***,$$$
AAA	137.6 ± 3.7	308.3 ± 6.4***	190.1 ± 6.4***,$$$	193.8 ± 2.1***,$$$	189.8 ± 9.2***,$$$
Fischer's ratio	4.1 ± 0.2	2.4 ± 0.18***	2.76 ± 0.19	2.8 ± 0.5$	2.9 ± 0.21

***,$$$are significant at P < 0.001; **,$$ are significant at P < 0.01; *,$ are significant at P < 0.05. Data are expressed as mean ± SD; n = 10. * Significantly different from control group. $ Significantly different from synfat-treated group.

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in death [32]. This study assessed QUR and MEX's anti-hypoxic potential in comparison to synfat-induced brain damage in the hypoxic group.

Data from the current study (Table 1) showed that rats intoxicated with synfat had a significantly lower Hb level than the control value. On the other hand, the Hb level was considerably returned to the control value by QUR, MEX, and the combined approach. According to Fadda et al. [33], synfat-conjugation to oxy-Hb breaks the bounded O_2 and creates meth-Hb, hydrogen peroxide, and nitric dioxide, which is the first step in a chain reaction involving free radicals [34]. Nitric dioxide oxidizes ferrous hemoglobin to methemoglobin, while hydrogen peroxide oxidizes meth-Hb to ferryl-Hb radical. Ferryl-Hb and nitrite react to produce

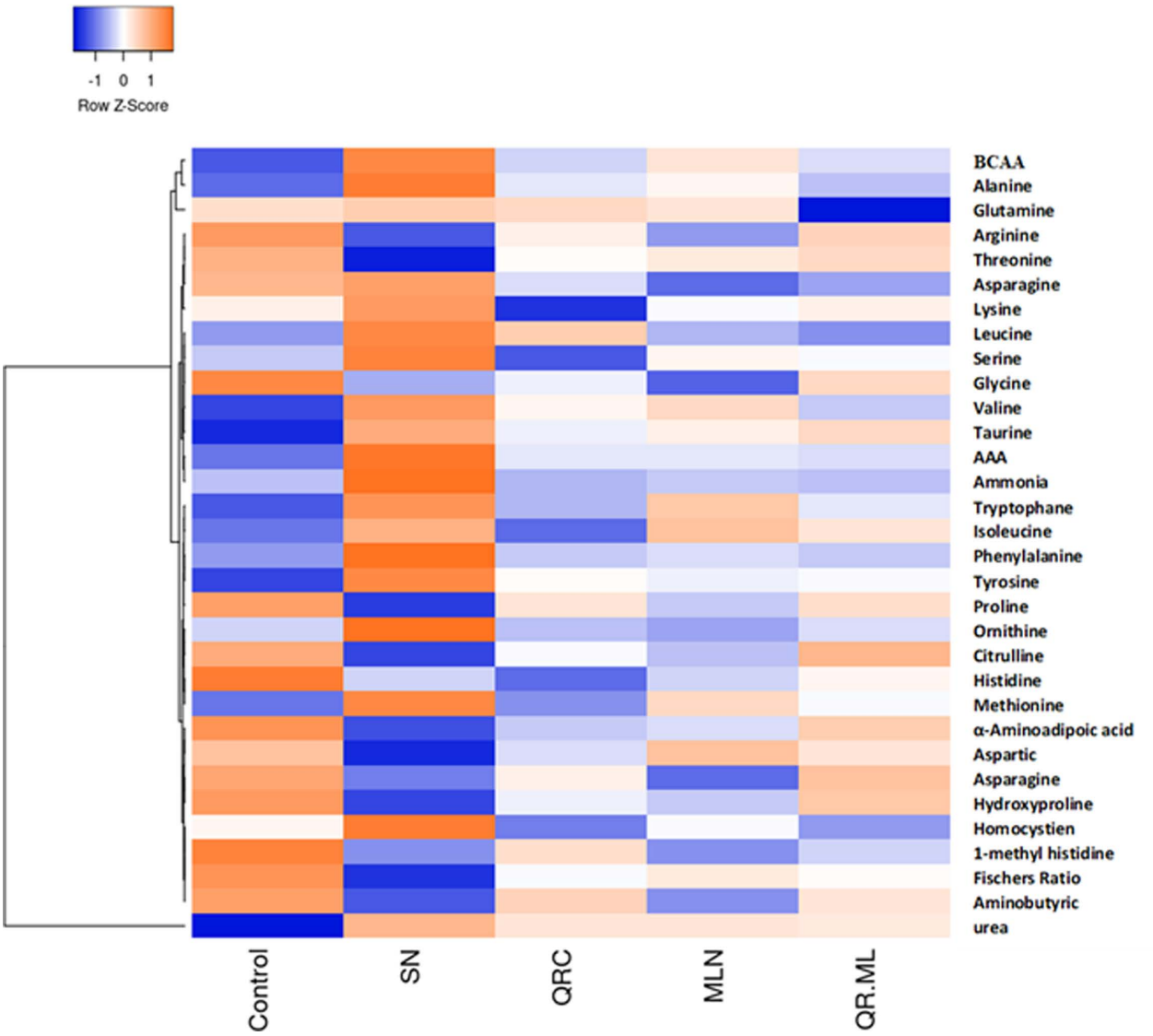

Fig 8. Heatmap representing different amino acids exists in synfat, QUR, MLN (Mexamine) and combination group. Orange represents high score while blue represents low score.

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met-Hb and NO2. Consequently, this prevents O_2-binding to Hb, which lowers blood's capability to conjugate O_2 and promotes hypoxia. By increasing lipid peroxidation and inhibiting antioxidant enzymes, hypoxemia causes oxidative stress in the brain [35]. In reaction to oxygen deletion and ROS production, synfat causes oxidative damage, reduces energy production, and impairs brain function [36]. Under the influence of oxidative stress, erythrocytes absorb MEX in order to produce heme and delay hemoglobin denaturation [37]. Similarly, QUR's anti-inflammatory and anti-oxidant properties are essential to its therapeutic effects.

By blocking the actions of xanthine oxidase and NADPH oxidase, QRC inhibits Nrf2 nuclear translocation, promotes the antioxidant cascade, and increases ROS [38]. OS, synfat toxic effects are also associated with inflammation [39].

Synfat here clarified an inflammatory response that was validated by the release of pro-inflammatory cytokines like TNF-α, CRP, and IL-6 (Fig.1). Activated macrophages produce

TNF-α, which plays a strong role in inflammation [40]. Cell necrosis is a result of synfat's damage to cells, DNA, and cell membranes [41]. Other studies of elevated TNF-α and IL-6 levels in rats exhibiting hypoxemia environments support our findings [42]. Brain injury is linked to the activation of NF-kB, which in turn causes the release of TNF-α and IL-6 [43]. However, administration of the antioxidants under investigation, prior to hypoxia induction, markedly reduced pro-inflammatory mediators triggered by hypoxemia, such as TNF-a, IL-6, and CRP. The immunomodulatory and anti-inflammatory properties of QUR and MEX are closely linked to their positive effects. The decrease of these mediators may provide protection against the inflammation that is exacerbated by different pathological conditions [44]. In hypoxic animals, MEX reduces inflammation by blocking the Nrf2 and NF-kB cascades as well as pro-inflammatory cytokines TNF-α and IL-6 [45]. Scavenging ROS and RNS, QUR capability demonstrates its antioxidant capacity. In addition, its ability to relax smooth muscle leads to the raises of the e-NO level with subsequent vasodilation, which in turn causes angiogenesis and vasodilation after hypoxemia [46]. By inhibiting apoptosis and stopping the mitochondria-dependent caspase cascade, QUR prevents ischemia-reperfusion death [47].

Neurotransmitters play a crucial part in regulating healthy physiological processes. The production of neurotransmitters is regulated by O_2-requiring rate-limiting enzymes. Hypoxemia may therefore affect neuronal signaling through the modulation of neurotransmitters [48].

Neurodegenerative diseases cause a decrease in serotonine levels and brain catecholamines [49]. In line with earlier studies, a decrease in serotonine, noradrenaline, and intropin levels was observed in the hypoxemia-triggered cerebral injury model (Fig. 2). This can be linked to the action of alleviated monoamine oxidase-A, which is activated via synfat and starts the breakdown and reduction of brain monoamines [50]. Similarly, hypoxemia reduces the activity of two key enzymes that play a crucial role in the production of serotonin and catecholamines: tryptophan hydroxylase and tyrosine hydroxylase [51].

Excessive glutamate production that contributes to neuronal damage is explained by hypoxia [52]. γ-aminobutyric acid counteracts brain toxicity and has neuroprotective effects [53]. Thereby, in the hypoxemic model, the observed decrease in GABA in this study can be attributed to a brain deficit. By blocking the GABA-synthetic pathway and lowering glutamate decarboxylase expression, GABA was markedly reduced in the brain stem and cerebellum of hypoxic neonatal rats, which is consistent with the decreased GABA in this hypoxemic model [54]. In comparison to synfat-intoxicated rats, QUR, MEX, and the combined therapy significantly triggered the decline in neurotransmitter levels. Melatonin has been shown to have protective effects on mitochondria, increasing calcium influx, producing ATP, and lowering oxidative stress [55]. Melatonin increases monoamine synthesis, which affects striatal dopamine levels [56]. In the striatum, QUR changed the neurotransmitters serotonine, dopamine, noradrenaline, and GABA.

The elevation in neurotransmitter levels is associated with either the QUR inhibitory effect or the inhibition of neuron oxidative strain [57].

The illustrative data in Tables 2 demonstrated that while Fischer'

s ratio decreased in rats intoxicated with sodium nitrite, the levels of AAAs and BCAAs were elevated. Rats treated with QUR and MEX showed a higher Fischer'.

s ratio, but their levels of BCAAs (leucine and isoleucine) and AAAs (phenylalanine and tyrosine) were lower. These findings were consistent with Pany et al'.

s data [58]. Tyrosine and phenylalanine metabolism in the liver contributes to the notable increase in AAAs as a result of the decline in hepatic metabolic function following hypoxemia [18]. The majority of muscle mass that produces ATP is produced by BCAAs [16].

The use of BCAAs is impacted by hypoxia based on the mitochondria's impaired electron transport system. Rats with anemia had higher levels of urea and free amino acids.

Furthermore, there was an increase in plasma levels of glutamine, alanine, phenylalanine, and tyrosine [58].

According to the current study, rats intoxicated with sodium nitrite showed decreased plasma arginine and citrulline concentrations, indicating a variation in the urea-cycle that depends on ATP release, which was reduced following synfat intoxication. Following QUR and MEX, levels of citrulline and arginine improved. Therapy. Similar results were found with Zunić et al [59].

Proline production was hampered by hypoxemia's reduction of plasma proline concentration, which led to ATP-diminution and a subsequent drop in γ-glutamyl kinase. Conversely, QUR and MEX increased the concentration of proline. This decrease in proline and citrulline concentrations was indicative of ischemic hypoxia [60].

In hypoxia, glutamate transport and metabolism underwent a radical shift, and the concentration of glutamate outside cells decreased [61]. According to additional findings, GABA receptors decreased under hypoxic conditions [62]. Similar results were found in the current data; glutamate and GABA concentrations increased despite the fact that the antioxidant treatment under investigation modulated these changes.

In this article, synfat-intoxication significantly triggered DNA damage, oxidative stress and DNA triggered oxidative stress biomarker 8-oxodG (Figs 3 –6). The reaction between NO and O2.-, which produces the highly reactive ONOO – (peroxynitrite) radicals, may shed light on this. Nitrotyrosine is a marker of nitrosative stress and is produced by ONOO – conjugating with proteins. Nitrotyrosine combines with DNA, phospholipids, and proteins to form products that cause disease. It also has a variety of mutagenic, genotoxic, and cytotoxic effects, including inhibiting the synthesis of DNA and proteins and deactivating enzymes. There are several ways in which sodium nitrite can cause DNA damage. Free radicals that cause chemical changes or ROS that start lipid peroxidation [63]. Neglected oxidative DNA damage triggers mutations or alterations in the cell's genetic material, which can result in apoptosis, neurodegeneration, chronic inflammation, carcinogens, altered cellular behavior, changes in gene expression, and genomic instability. As a result, oxidative DNA damage is linked to a number of diseases, including diabetes, cancer, neurodegenerative, cardiovascular, and autoimmune inflammatory diseases, as well as other age-related conditions.

In order to introduce the altered bases into the genomic DNA during replication or repair, ROS can oxidize the DNA bases directly in the genomic DNA strands or indirectly in the nucleotide pool. The ability of 8-oxoG to form Watson–Crick base pairs with Cytosine, which results in G:C–C:G transversions, and Hoogsteen base pairs with Adenine, which results in G:C –T:A transversions during DNA replication, is linked to its high mutagenic capacity.

Accordingly, there has been growing evidence of the potential evaluation of 8-oxoG and/or 8-oxodG in blood, saliva and tissue, and they are thought to be the principal biomarkers for oxidative-DNA damage. In addition, they are utilized to assess the body's oxidative stress as well as to assess the risk, diagnose, and forecast the therapeutic index for a number of illnesses.

Conversely, oxidative stress and brain DNA damage were effectively reduced by QUR and/or MEX treatment. The aforementioned findings support those of Rathi et al. [64], who found that mexamine enhances brain oxidative stress biomarkers that contribute to DNA damage and quercetin has anti-inflammatory, antiviral, and antitumor effects. Similarly, mexamine's ability to scavenge free radicals offers a defense mechanism against oxidative and DNA damage [65].

Conclusion

QUR and MEX combined therapy revealed a prospective therapeutic impact against synfat triggered brain injury via modulating inflammatory cytokines including CRP, IL-6 and TNF-α in addition to, neurotransmitters (Intropin, serotonine, noradrenaline and GABA)

furthermore, DNA damage, 8-Oxodg and histomorphorological investigations confirmed these findings.

Study limitation

Animals count caused some limitations in the study. Synfat handling may lead to asthma with breath shortness, and chest tightness. Animal models don't perfectly resemble the way that the human body and disorder may respond to treatments.

Author contributions

Data curation: Mai O. Kadry, Hanaa Ali.

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Investigation: Mai O. Kadry, Hanaa Ali.

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Visualization: Mai O. Kadry, Hanaa Ali.

Writing – original draft: Mai O. Kadry, Hanaa Ali.

Writing – review & editing: Mai O. Kadry, Hanaa Ali.

References

1. Yang G, Shi R, Zhang Q. Hypoxia and oxygen-sensing signaling in gene regulation and cancer progression. Int J Mol Sci. 2020;21(21):8162. https://doi.org/10.3390/ijms21218162 PMID: 33142830
2. Timilsina A, Dong W, Hasanuzzaman M, Liu B, Hu C. Nitrate-nitrite-nitric oxide pathway: a mechanism of hypoxia and anoxia tolerance in plants. Int J Mol Sci. 2022;23(19):11522. https://doi.org/10.3390/ijms231911522 PMID: 36232819
3. Salama MF, Abbas A, Darweish MM, El-Hawwary AA, Al-Gayyar MMH. Hepatoprotective effects of cod liver oil against sodium nitrite toxicity in rats. Pharm Biol. 2013;51(11):1435–43. https://doi.org/10.3109/13880209.2013.796564 PMID: 23862714
4. Karwowska M, Kononiuk A. Nitrates/Nitrites in food-risk for nitrosative stress and benefits. Antioxidants (Basel). 2020;9(3):241. https://doi.org/10.3390/antiox9030241 PMID: 32188080
5. Lan K-M, Tien L-T, Cai Z, Lin S, Pang Y, Tanaka S, et al. Erythropoietin ameliorates neonatal hypoxia-ischemia-induced neurobehavioral deficits, neuroinflammation, and hippocampal injury in the juvenile rat. Int J Mol Sci. 2016;17(3):289. https://doi.org/10.3390/ijms17030289 PMID: 26927081
6. Aita NA, Mohammed FF. Effect of marjoram oil on the clinicopathological, cytogenetic and histopathological alterations induced by sodium nitrite toxicity in rats. Glob Vet. 2014;12:606–16.
7. Sataieva T, Zadnipryany I. Hypoxic damage of cardiomyocytes during pregnancy and its experimental treatment. Int Stud J Med. 2015;1(1):18–21.
8. Petrova E, Gluhcheva Y, Pavlova E, Vladov I, Voyslavov T, Ivanova J. Effect of acute sodium nitrite intoxication on some essential biometals in mouse spleen. J Trace Elem Med Biol. 2020;58:126431. https://doi.org/10.1016/j.jtemb.2019.126431 PMID: 31759232
9. S K S S, , P H, Mathew T, S S, M C. Nifedipine inhibits hypoxia induced transvascular leakage through down regulation of NFkB. Respir Physiol Neurobiol. 2012;183(1):26–34. https://doi.org/10.1016/j.resp.2012.05.016 PMID: 22627105
10. Ghosh D, Levault KR, Brewer GJ. Relative importance of redox buffers GSH and NAD(P)H in age-related neurodegeneration and Alzheimer disease-like mouse neurons. Aging Cell. 2014;13(4):631–40. https://doi.org/10.1111/acel.12216 PMID: 24655393
11. Yadav RS, Shukla RK, Sankhwar ML, Patel DK, Ansari RW, Pant AB, et al. Neuroprotective effect of curcumin in arsenic-induced neurotoxicity in rats. Neurotoxicology. 2010;31(5):533–9. https://doi.org/10.1016/j.neuro.2010.05.001 PMID: 20466022
12. Kumar GK. Hypoxia. 3. Hypoxia and neurotransmitter synthesis. Am J Physiol Cell Physiol. 2011;300(4):C743–51. https://doi.org/10.1152/ajpcell.00019.2011 PMID: 21270298

13. Grosenbaugh DK, Ross BM, Wagley P, Zanelli SA. The role of kainate receptors in the pathophysiology of hypoxia-induced seizures in the neonatal mouse. Sci Rep. 2018;8(1):7035. https://doi.org/10.1038/s41598-018-24722-3 PMID: 29728616

14. Attia H, Fadda L, Al-Rasheed N, Al-Rasheed N, Maysarah N. Carnosine and L-arginine attenuate the downregulation of brain monoamines and gamma aminobutyric acid; reverse apoptosis and upregulate the expression of angiogenic factors in a model of hemic hypoxia in rats. Naunyn Schmiedebergs Arch Pharmacol. 2020;393(3):381–94. https://doi.org/10.1007/s00210-019-01738-8 PMID: 31641819

15. Hilaire G, Voituron N, Menuet C, Ichiyama R, Subramanian H, Dutschmann M. Role of serotonin in respiratory function and dysfunction. Respir Physiol Neurobiol. 2010;174(1):76–88.

16. Al-Rasheed NM, Fadda LM, Al-Rasheed NM, Attia H, Ali HM, El-Agami H. Role of natural antioxidants in the modulation of plasma amino acid pattern in rats exposed to hemic hypoxia. Braz Arch Biol Technol. 2015;58(5):741–9.

17. Muratsubaki H, Yamaki A. Profile of plasma amino Acid levels in rats exposed to acute hypoxic hypoxia. Indian J Clin Biochem. 2011;26(4):416–9. https://doi.org/10.1007/s12291-011-0125-3 PMID: 23024481

18. Zenlander R, Fredolini C, Schwenk JM, Rydén I, Påhlsson P, Löwbeer C, et al. A wide scan of plasma proteins demonstrates thioredoxin reductase 1 as a potential new diagnostic biomarker for hepatocellular carcinoma. Scand J Gastroenterol. 2023;58(9):998–1008. https://doi.org/10.1080/00365521.2023.2194008 PMID: 37017178

19. Fadda LM, Attia HA, Al-Rasheed NM, Ali HM, Al-Rasheed NM. Roles of some antioxidants in modulation of cardiac myopathy induced by sodium nitrite via down-regulation of mRNA expression of NF-κB, Bax, and flt-1 and suppressing DNA damage. Saudi Pharm J. 2018;26(2):217–23. https://doi.org/10.1016/j.jsps.2017.12.008 PMID: 30166919

20. Alshanwani AR, Shaheen S, Faddah LM, Alhusaini AM, Ali HM, Hasan I, et al. Manipulation of quercetin and melatonin in the down-regulation of HIF-1α, HSP-70 and VEGF pathways in rat's kidneys induced by hypoxic stress. Dose-Response. 2020;18(3):1559325820949797. https://doi.org/10.1177/1559325820949797

21. Leung JW-H, Cheung K-K, Ngai SP-C, Tsang HW-H, Lau BW-M. Protective effects of melatonin on neurogenesis impairment in neurological disorders and its relevant molecular mechanisms. Int J Mol Sci. 2020;21(21):5645. https://doi.org/10.3390/ijms21215645

22. Tripathi A, Kumar B, Sagi SSK. Hypoxia-mediated alterations in pulmonary surfactant protein expressions: Beneficial effects of quercetin prophylaxis. Respir Physiol Neurobiol. 2021;291:103695. https://doi.org/10.1016/j.resp.2021.103695 PMID: 34052411

23. Huang R, Zhong T, Wu H. Quercetin protects against lipopolysaccharide-induced acute lung injury in rats through suppression of inflammation and oxidative stress. Arch Med Sci. 2015;11(2):427–32. https://doi.org/10.5114/aoms.2015.50975 PMID: 25995762

24. Hoque ME, Shanta SM, Tahrin R, Chowdhury S, Tasnim Z, Shuvo MAA, et al. A benign way of measuring hemoglobin in blood – Towards developing a non-invasive technique. Hybrid Advances. 2023;3:100039. https://doi.org/10.1016/j.hybadv.2023.100039

25. Kadry MO. Liposomal glutathione as a promising candidate for immunological rheumatoid arthritis therapy. Heliyon. 2019;5(7):e02162. https://doi.org/10.1016/j.heliyon.2019.e02162 PMID: 31384691

26. Ranjbar-Slamloo Y, Fazlali Z. Dopamine and noradrenaline in the brain; overlapping or dissociate functions?. Front Mol Neurosci. 2020;12:334. https://doi.org/10.3389/fnmol.2019.00334 PMID: 32038164

27. Jie F, Yin G, Yang W, Yang M, Gao S, Lv J, et al. Stress in regulation of GABA amygdala system and relevance to neuropsychiatric diseases. Front Neurosci. 2018;12:562. https://doi.org/10.3389/fnins.2018.00562 PMID: 30154693

28. Ji Y, Karbaschi M, Abdulwahed A, Quinete NS, Evans MD, Cooke MS. A High-throughput comet assay approach for assessing cellular DNA damage. J Vis Exp. 2022;(183):10.3791/63559. https://doi.org/10.3791/63559 PMID: 35635461

29. Lopez MJ, Mohiuddin SS. Biochemistry, Essential Amino Acids. National library of medicine; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557845/

30. Li K, Zheng Y, Wang X. The potential relationship between HIF-1α and amino acid metabolism after hypoxic ischemia and dual effects on neurons. Front Neurosci. 2021;15:676553. https://doi.org/10.3389/fnins.2021.676553 PMID: 34483819

31. Li D, Ren J, Du Q, Liu P, Li Y. The anti-hypoxic effects of oat (Avena sativa L.) oligopeptides in mice. Am J Transl Res. 2021;13(3):1657–66. PMID: 33841687

32. Ansari FA, Mahmood R. Sodium nitrite enhances generation of reactive oxygen species that decrease antioxidant power and inhibit plasma membrane redox system of human erythrocytes. Cell Biol Int. 2016;40(8):887–94. https://doi.org/10.1002/cbin.10628 PMID: 27214747

33. Fadda LM, Attia HA, Al-Rasheed NM, Ali HM, Al-Rasheed NM. Downregulation of flt-1 and HIF-1α gene expression by some antioxidants in rats under sodium nitrite-induced hypoxic stress. Dose Response. 2018;16(2):1559325818776204. https://doi.org/10.1177/1559325818776204 PMID: 29872369

34. Kadry MO, Ali HM. Downregulation of HIF1-α, Smad-2, AKT, and Bax gene expression in sodium nitrite-induced lung injury via some antioxidants. J Biochem Mol Toxicol. 2017;31(7).

35. David SR, Sawal NS, Hamzah MNSB, Rajabalaya R. The blood blues: a review on methemoglobinemia. J Pharmacol Pharmacotherapeutics. 2018;9(1):1–5. https://doi.org/10.4103/jpp.jpp_79_17

36. Wang C, Yan M, Jiang H, Wang Q, He S, Chen J, et al. Mechanism of aquaporin 4 (AQP 4) up-regulation in rat cerebral edema under hypobaric hypoxia and the preventative effect of puerarin. Life Sci. 2018;193:270–81. https://doi.org/10.1016/j.lfs.2017.10.021 PMID: 29054452

37. Al-Rasheed NM, Fadda L, Attia HA, Sharaf IA, Mohamed AM, Al-Rasheed NM. Original research paper. Pulmonary prophylactic impact of melatonin and/or quercetin: A novel therapy for inflammatory hypoxic stress in rats. Acta Pharm. 2017;67(1):125–35. https://doi.org/10.1515/acph-2017-0010 PMID: 28231050

38. Singh S, Jamwal S, Kumar P. Neuroprotective potential of Quercetin in combination with piperine against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. Neural Regen Res. 2017;12(7):1137–44. https://doi.org/10.4103/1673-5374.211194 PMID: 28852397

39. Carrasco-Pozo C, Castillo RL, Beltrán C, Miranda A, Fuentes J, Gotteland M. Molecular mechanisms of gastrointestinal protection by quercetin against indomethacin-induced damage: role of NF-κB and Nrf2. J Nutr Biochem. 2016;27:289–98. https://doi.org/10.1016/j.jnutbio.2015.09.016 PMID: 26507542

40. Bai W, Zhou J, Zhou N, Liu Q, Cui J, Zou W, et al. Hypoxia increased RAGE expression regulates chemotaxis and proinflammatory cytokines release through nuclear translocation of NF-κB and HIF1α in THP-1 cells. Biochem Biophys Res Commun. 2018;495(3):2282–8.

41. Davis Sanders O, Rajagopal L, Chase Barton C, Archa Rajagopal J, Lopez O, Lopez K, et al. Does oxidative DNA damage trigger histotoxic hypoxia via PARP1/AMP-driven mitochondrial ADP depletion-induced ATP synthase inhibition in Alzheimer's disease?. Mitochondrion. 2022;67:59–64. https://doi.org/10.1016/j.mito.2022.10.005 PMID: 36367519

42. Hanumanthachar P, Sandhya C, Charan C. Cerebroprotective effects of D. triquetrum on sodium nitrite-induced hypoxia and ethanol-induced neurodegeneration in animal models relevant to dementia. Alzheimer's Dement. 2020;16(Suppl. 9):e041970.

43. Yoo Y-M, Joo SS. Melatonin can modulate neurodegenerative diseases by regulating endoplasmic reticulum stress. Int J Mol Sci. 2023;24(3):2381. https://doi.org/10.3390/ijms24032381 PMID: 36768703

44. Aghababaei F, Hadidi M. Recent advances in potential health benefits of quercetin. Pharmaceuticals (Basel). 2023;16(7):1020. https://doi.org/10.3390/ph16071020 PMID: 37513932

45. Hassell KJ, Reiter RJ, Robertson NJ. Melatonin and its role in neurodevelopment during the perinatal period: a review. Fet Matern Med Rev. 2013;24(2):76–107. https://doi.org/10.1017/s0965539513000089

46. Chakraborty J, Singh R, Dutta D, Naskar A, Rajamma U, Mohanakumar KP. Quercetin improves behavioral deficiencies, restores astrocytes and microglia, and reduces serotonin metabolism in 3-nitropropionic acid-induced rat model of Huntington's Disease. CNS Neurosci Ther. 2014;20(1):10–9. https://doi.org/10.1111/cns.12189 PMID: 24188794

47. Wu Y, Wei H, Li P, Zhao H, Li R, Yang F. Quercetin administration following hypoxia-induced neonatal brain damage attenuates later-life seizure susceptibility and anxiety-related behavior: modulating inflammatory response. Front Pediatr. 2022;10:791815. https://doi.org/10.3389/fped.2022.791815 PMID: 35223693

48. Cools R. Role of dopamine in the motivational and cognitive control of behavior. Neuroscientist. 2008;14(4):381–95. https://doi.org/10.1177/1073858408317009 PMID: 18660464

49. Tozihi M, Shademan B, Yousefi H, Avci CB, Nourazarian A, Dehghan G. Melatonin: a promising neuroprotective agent for cerebral ischemia-reperfusion injury. Front Aging Neurosci. 2023;15:1227513. https://doi.org/10.3389/fnagi.2023.1227513 PMID: 37600520

50. Shrivastava V, Dey D, Singal CMS, Jaiswal P, Singh A, Sharma JB, et al. Glutamate uptake is not impaired by hypoxia in a culture model of human fetal neural stem cell-derived astrocytes. Genes (Basel). 2022;13(3):506. https://doi.org/10.3390/genes13030506 PMID: 35328060

51. Kumar A, Gupta M, Sharma R, Sharma N. Deltamethrin-induced immunotoxicity and its protection by quercetin: an Experimental Study. Endocr Metab Immune Disord Drug Targets. 2020;20(1):67–76. https://doi.org/10.2174/1871530319666190410144540 PMID: 30968779

52. Yang T, Zhang X-M, Tarnawski L, Peleli M, Zhuge Z, Terrando N, et al. Dietary nitrate attenuates renal ischemia-reperfusion injuries by modulation of immune responses and reduction of oxidative stress. Redox Biol. 2017;13:320–30. https://doi.org/10.1016/j.redox.2017.06.002 PMID: 28623824

53. Son J, Baritugo K-A, Sohn YJ, Kang KH, Kim HT, Joo JC, et al. Production of γ-Aminobutyrate (GABA) in recombinant corynebacterium glutamicum by expression of glutamate decarboxylase active at neutral pH. ACS Omega. 2022;7(33):29106–15. https://doi.org/10.1021/acsomega.2c02971 PMID: 36033683

54. Bassetti D. Keeping the balance: GABAB receptors in the developing brain and beyond. Brain Sci. 2022;12(4):419. https://doi.org/10.3390/brainsci12040419 PMID: 35447949

55. Spasojevic N, Stefanovic B, Jovanovic P, Dronjak S. Anxiety and hyperlocomotion induced by chronic unpredictable mild stress can be moderated with melatonin treatment. Folia Biol (Praha). 2016;62(6):250–7. https://doi.org/10.14712/fb2016062060250 PMID: 28189148

56. Hoehn R, Monse M, Pohl E, Wranik S, Wilker B, Keitsch S, et al. Melatonin acts as an antidepressant by inhibition of the acid sphingomyelinase/ceramide system. Neurosignals. 2016;24(1):48–58. https://doi.org/10.1159/000442611 PMID: 27398923

57. Al rasheed N, Fadda L, Attia H, Ali H, Al-Rasheed N. Quercetin inhibits sodium nitrite-induced inflammation and apoptosis in different rats organs by suppressing Bax, HIF1-α, TGF-β, Smad-2, and AKT pathways. J biochem Mol toxicol. 2016;31(5):.

58. Pany S, Pal A, Sahu P. Neuroprotective effect of quercetin in neurotoxicity induced rats: role of neuroinflammation in neurodegeneration. Asian J Pharm Clin Res. 2014;7:152–6.

59. Lee N, Kim D. Toxic metabolites and inborn errors of amino acid metabolism: what one informs about the other. Metabolites. 2022;12(6):527. https://doi.org/10.3390/metabo12060527 PMID: 35736461

60. Park K-T, Han J-K, Kim SJ, Lim Y-H. Gamma-aminobutyric acid increases erythropoietin by activation of citrate cycle and stimulation of hypoxia-inducible factors expression in rats. Biomolecules. 2020;10(4):595. https://doi.org/10.3390/biom10040595 PMID: 32290638

61. Kaity S, Kumar A. Antioxidants in brain injury with or without antibiotics. Vitamins and Minerals in Neurological Disorders. 2023.137–56.

62. Zhang C, He J, Wang X, Yang Y, Huang Q, Qiao F, et al. Gamma-aminobutyric acid enhances hypoxia tolerance of juvenile Chinese mitten crab (Eriocheir sinensis) by regulating respiratory metabolism and alleviating neural excitotoxicity. Comp Biochem Physiol C Toxicol Pharmacol. 2022;260:109409. https://doi.org/10.1016/j.cbpc.2022.109409 PMID: 35830953

63. Rana A, Singh S, Sharma R, Kumar A. Traumatic brain injury altered normal brain signaling pathways: implications for novel therapeutics approaches. Curr Neuropharmacol. 2019;17(7):614–29. https://doi.org/10.2174/1570159X16666180911121847 PMID: 30207236

64. Rathi V, Tiwari I, Kulshreshtha R, S K Sagi S. Hypobaric hypoxia induced renal injury in rats: Prophylactic amelioration by quercetin supplementation. PLoS One. 2023;18(2):e0279304. https://doi.org/10.1371/journal.pone.0279304 PMID: 36827356

65. Singhanat K, Apaijai N, Sumneang N, Maneechote C, Arunsak B, Chunchai T, et al. Therapeutic potential of a single-dose melatonin in the attenuation of cardiac ischemia/reperfusion injury in prediabetic obese rats. Cell Mol Life Sci. 2022;79(6):300. https://doi.org/10.1007/s00018-022-04330-1 PMID: 35588335