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Original Article

Antioxidant properties of morin against arsenic neurotoxicity in rats showed ameliorative effects on pain and inflammation

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ABSTRACT

Experimental evidence has demonstrated that arsenic exposure, whether acute or chronic, is detrimental to all systems of the body. This study aims to investigate whether morin could decrease the neurotoxic impact of arsenic on Wistar rats concerning pain and inflammation. Arsenic (20 mg/kg body Wt.) was administered orally to Wistar rats. We kept one group of animals on drinking water, and the other group received treatment with morin (50 mg/kg body Wt.).The 28-day experiment was conducted weekly, i.e., on the 7th, 14th, 21st, and 28th day. Nociceptive pain was measured by the tail flick test (TFT) and hot plate test (HPT), while inflammatory pain was measured by Randal pain test (RPT). On the 28th day, animals were sacrificed. Catalase, superoxide dismutase activity, lipid peroxidation and glutathione (GSH) were measured in the brain tissue. Histological alterations in the cerebral cortex and hippocampus were studied using Congo red stain. Morin showed a significant antinociceptive effect as evidenced by TFT and HPT and an anti-inflammatory effect as demonstrated by RPT. Arsenic increased lipid peroxidation and inhibited the activities of superoxide dismutase, glutathione content and catalase simultaneously. The administration of morin suppressed lipid peroxidation while concurrently increasing the activities of catalase and superoxide dismutase and the glutathione content. Conversely, morin ameliorated the alterations in cerebral cortex and hippocampal histology in arsenic-poisoned rats. Consequently, morin showed antinociceptive, anti-inflammatory, and antioxidant qualities against arsenic-induced neurotoxicity in rats.

KEYWORDS: arsenic, morin, cerebral cortex, hippocampus, catalase, superoxide dismutase, lipid peroxidation.

1. INTRODUCTION

Arsenic, the 20th most common element in nature, is present in the biosphere and the world's crust. When rocks weather, arsenic becomes incorporated into the soil and groundwater through leaching and runoff. Anthropogenic activities can also introduce it into soil and groundwater [1]. The main ways that humans are exposed to arsenic are through tainted drinking water; skin absorption and through inhalation, which is slightly less common [2]. Chronic human exposure to arsenic through drinking water causes almost all organs to suffer from cancer, skin diseases (including hyperpigmentation and hyperkeratosis) that lead to dermal and epithelial tissue cancers, challenges to organs such as the kidneys, liver, heart, brain, and reproductive organs, intestinal issues and intellectual impairment in children. These ailments increase morbidity and mortality [3, 4]. Since there is no specific, secure, or effective treatment for arsenicosis, arsenic poisoning is regarded as a significant issue on a global scale. Thus, it is evident that an efficient treatment for arsenicosis is required [4, 5]. As of now, there is no reliable, regularly effective treatment for arsenicosis.

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Antioxidant therapy and vitamin and mineral supplements are recommended treatment alternatives [5]. Since the earliest days of humankind, plants and plant-derived products have been used to alleviate illness. The main advantages of plant-based therapy are its low cost, absence of severe side effects, and efficacy [6]. According to a literature study, research has been undertaken over the last ten years to uncover therapeutic plants and natural components, or phytochemicals that may be capable of avoiding arsenic toxicity in people and animals. In animal models, several medicinal herbs and phytochemicals demonstrated an intense protective effect against arsenic toxicity caused through experimentation.

There is evidence that exposure to arsenic can be potentially dangerous to the nervous system. However, the research on this topic could be more extensive [7]. The most hazardous inorganic arsenic specifically damages voltage-gated potassium channels and impairs cell electrolysis, which can lead to neurological disorders, cardiovascular issues, nervous system dysfunction, and other issues. Heavy metal exposure in the environment is a contributing factor in neuropathological damage and cognitive decline. Arsenic suppresses the growth of neural progenitor cells and reduces neuronal migration and cellular maturation [8]. The reaction of arsenic with sulfhydryl groups has significant toxicity and harmful effects on the metabolism of proteins [9], which explains why arsenic exposure lowers the body's level of free thiol, a powerful antioxidant.

Arsenic causes cognitive problems, which lead to behavioural abnormalities. Arsenic (As) exposure disrupts the central nervous system and impairs learning and memory. Catecholamines, including norepinephrine (NE), dopamine (DA), and serotonin (5-HT), are neurotransmitters essential for memory and learning. The majority of neurotoxic toxicants, including arsenic, are inflammatory, and they all share one thing in common: prostaglandin release [10]. The overexpression of inducible cyclooxygenase 2 (COX-2), the enzyme in charge of PG production, could be a contributing factor to the worsening of the inflammatory state [11]. However, PGs are inflammatory solid mediators; they also cause pain signals to be generated and amplified at the peripheral and spinal levels, which affects nociception [12].

Various studies have tested the toxicity of arsenic in numerous therapeutic plants. The effect of plants such as *Withania somnifera* roots were tested on the testes, liver, and kidneys of arsenic toxicity-induced rats [13]. The effect of *Jpomea aquatica* aerial parts were tested on the testes, brain, heart, kidney and liver of arsenic toxicity-induced mice [14]. Das *et al*., 2010 [15] tested the ameliorative effect of *Corchorus olitorius* leaves on the brains of rats. Additionally, various studies have investigated a range of artificial and biological compounds that can act against the toxicity of arsenic both *in vivo* and *in vitro*. Rutin was evaluated for behavioral and electrophysiological changes in rats with arsenic poisoning and is one of the natural products proven to have ameliorative potential [16]. Queretin is another isolated substance that can protect the liver, brain, and testicles in arsenic-treated rats [17]. The effect of curcumin on the liver and brain of arsenic toxicity-induced rats has also been evaluated [18]. The majority of research indicates that plants and phytochemicals of plants play a protective function in lowering arsenic-induced toxicity, as evidenced by improvements in oxidative stress indices.

Researchers have examined many plants for the presence of phytochemicals that may have medicinal applications, such as flavonoids, carotenes, saponins, polyphenols, and flavonols. Compared to synthetic medications, these extracts have fewer side effects and are less toxic. Fruits and vegetables are rich in flavonoids, which are polyphenolic secondary plant metabolites. Flavonoids have numerous pharmacological actions [19]. Flavonols are flavonoids with a ketone group. They are proanthocyanin building blocks. Many different fruits and vegetables contain flavonols. Morin is a flavanol extracted from several plants, most notably from the Moraceae family. Other natural sources of morin are almonds [20], guava [21], old fustic [22], osage orange [23], *Acridocarpus orientalis* [24], onion (*Allium cepa*) [25], apple (*Malus pumila*) [26], tea (*Camellia sinensis*) [27] and other beverages, red wine [28], coffee [29], cereal grain [30] etc. Morin is beneficial against a variety of neurological and other ailments, including dementia, Alzheimer's disease [31], Parkinson's disease [32], ischemia [33], diabetes [34], cancer [35], cardiovascular anomalies [36] and renal problems [37].

Therefore, we carried out this investigation to determine the protective role of morin against arsenic-induced oxidative stress, brain histological changes, and nociceptive and inflammatory alterations in rats.

2. MATERIALS AND METHODS

In this investigation, Wistar rats were used. Three-month-old rats from Jeeva Life Sciences (CPCSEA/ IAEC/JLS/16/07/21/44), Hyderabad, were used, and we kept the animals in standard lab conditions.

2.1. Experimental design

There are four groups of animals. The first group is control (C) which did not receive any specific treatment expect drinking water; the second group is 'As', wherein rats were treated with arsenic (20 mg/Kg BW); The third group is 'As+M' where arsenic-poisoned rats (dosage of 20 mg/kg body weight) were treated with morin (dosage of 50 mg/kg body weight); and the fourth group is 'M' where control rats were treated with morin (50 mg/kg bw). All animals were treated orally for 28 days.

2.2. Nociception, inflammatory and pain studies

2.2.1. Randall Selitto pain test

The pain response to mechanical stimulus was assessed by the Randal Selitto instrument each week during experimentation, according to Chipkin *et al*., 1983 [38].

2.2.2. Hot plate test

The hot plate test evaluated the nociceptive property of morin (Lavich *et al*., 2005) [39]. We maintained the Eddy hot plate instrument at 50-55 °C temperature. The sensitive reaction of animals to thermal pain, shown by licking their paws or jumping, was used to judge the nociception. The time was noted in seconds, and the cutoff time was fixed as 60 seconds to prevent damage.

2.2.3. Tail flick test

The tail-flick test evaluates the nociception of experimental animals according to the method of Amour D *et al*. (1941) [40]. A beaker full of water was heated and maintained at a temperature 50 °C. The tail end of the rat was immersed in the warm water till it shows a response in terms of a tail flick. We noted the time taken to flick in seconds. We did the experiment on the 7^{th}, 14^{th}, 21^{st} and 28^{th} days.

2.3. Neurobiochemical studies

2.3.1. Lipid peroxidation (LPO)

Malondialdehyde was estimated by the method of Wills, 1966 [41]. The results were analyzed using the SPSS software and expressed as nmol MDA/mg protein/3 mins.

2.3.2. Superoxide dismutase (SOD)

Superoxide dismutase enzyme activity was measured according to the method of Kono, 1978 [42].

$$2\ O_2 + 2\ H+ \text{ --SOD-->} H_2O_2 + O_2$$

It is a simple and rapid method for measuring superoxide in cytosol based on the enzyme's ability to prevent pyrogallol auto-oxidation. This technique relies on the enzyme's ability to suppress pyrogallol's oxygen-dependent auto-oxidation, or the auto-oxidation rate, by measuring the rise in absorbance at 420 nm.

2.3.4. Catalase

We measured catalase activity according to Luck 1971 [43].

2.3.5. Glutathione (GSH)

GSH was measured using the method of Irfan Rahman *et al.*, 2006 [44]. The final concentration of the reaction mixture contained 0.20 mM NADPH, 0.6 mM 5.5'-dithiobis-(2-nitrobenzoic acid), 0.5 units of glutathione reductase in 125 mM sodium phosphate buffer (pH 7.5), 6.3mM EDTA and 50 µl of sample volume. At 412 nm, the rate of reduction of 5.5'-dithiobis-(2-nitrobenzoic acid) was measured. GSH is expressed as n mole/mg protein.

2.4. Congo red stain for amyloid plaques

Done according to Luna, 1968 [45].

2.5. Statistical analysis

We used SPSS software for statistical analysis. We performed mean±SD (standard deviation), ±SE (standard error), multiple comparison tests, one-way ANOVA variance significance test ($p < 0.05$), multiple regression, post hoc multiple comparison

tests between experimental days, and Null hypothesis (H0). The data are presented in tables as mean±SE and percentage of variation between experimental groups in comparison of control group. Graphs were plotted using mean ± SD with origin 6.0 and Sigma Plot 9.0 software.

3. RESULTS

3.1. Randal pain test

Table 1 depicts the Randal pain test results. The arsenic-treated group (As) prolonged the paw withdrawal latency in comparison to the control (C) right from the first week to the seventh week, and the percentage of variation of the experimental group in comparison with the control was 45%, 83.8%, 97.0 % and 107.8% on the 7th, 14th, 21st and 28th day, respectively. Arsenic-poisoned rats treated with morin (As+M) showed significant latency recovery compared to Arsenic (As)-treated animals. The percentage of variation in comparison with the control is as follows: 7th day 20% and 38.7% on the 14th day, 52.9% on the 21st day, and 63.15% on the 28th day. The morin-treated control animals (M) showed same pain latency as that of control animals (C) (percentage of variation in comparison with control - 0% on 7th day 3.2% on 14th day, 5.8% on 21st day and 2.6% on 28th day). The results suggest the analgesic properties of morin.

3.2. Hot plate test

Table 2 displays the results of the hot plate test. The thermal latency of control animals (C) has increased significantly from the first week to the fourth week, and the percentage of variation of arsenic-treated animals in comparison with the control group is 51.85%, 1166%, 143.67%, and 159.8% on the 7th, 14th, 21st, and 28th day. Control and arsenic (As)-treated animals showed a significant latency difference. In arsenic-poisoned rats treated with morin (As+M), the difference in latency is substantial, but not as compared to control animals. The percentage of variation in comparison with control on the 7th day was 29.62% and 69.44% on the 14th day, 88.50% on the 21st day, and 119.60% on the 28th day. Morin-treated control animals (M) showed no significant difference from that of control animals (C) (the percentage of variation in comparison with control - 3.70% on the 7th day, 5.55% on the 14th day, 344% on 21st day and 3.921% on the 28th day).

Table 1. Effect of morin on mechanical stimulus of arsenic-treated rats.

Experimental Days/Groups	Group-I (Control)	Group-II (As)	Group-III (As+M)	Group-IV (M)	Percentage change over control		
					Group-II	Group-III	Group-IV
Day 7	0.20±0.007	0.29±0.006	0.24±0.005	0.20±0.008	45	20	0
Day 14	0.31±0.003	0.57±0.017	0.43±0.007	0.32±0.164	83.8	38.7	3.2
Day 21	0.34±0.005	0.67±0.022	0.52±0.011	0.36±0.013	97.0	52.9	5.8
Day 28	0.38±0.005	0.79±0.026	0.62±0.005	0.39±0.004	107.8	63.15	2.6

The values represent Mean ± SE and the percentage of variation between control and other experimental groups. All values are significant with P<0.05. Time was calculated in seconds, representing animals' resistance.

Table 2. Effect of morin on thermal latency of arsenic-treated rats.

Experimental Days/Groups	Group-I (Control)	Group-II (As)	Group-III (As+M)	Group-IV (M)	Percentage change over control		
					Group-II	Group-III	Group-IV
Day 7	10.8±0.374	16.4±0.6	14±1.870	11.2±0.374	51.85	29.62	3.70
Day 14	14.4±0.4	31.2±0.2	24.4±0.4	15.2±0.2	116.66	69.44	5.55
Day 21	17.4±0.6	42.4±0.678	32.8±0.583	18±0.547	143.67	88.50	3.44
Day 28	20.4±0.509	53±o.707	44.8±0.860	21.2±0.836	159.80	119.60	3.921

The values represent Mean ± SE and the percentage of variation between control and other experimental groups. All values are significant with P<0.05. The thermal latency is expressed in seconds.

3.3. Tail flick test

Figure 1 depicts the results of the tail flick test. Control animals (C) did not show any significant difference in response to tail flick throughout the four weeks of the study. Compared to control animals, Arsenic treated rats (As) demonstrated significant differences in tail flick reaction time, increasing from 1st to 4th week. The highest reaction time was 60 seconds for arsenic-treated animals (As), i.e. on the 4th week of study, and the percentage of variation in comparison with the control group was 44.77%, 106.57%, 161.36%, and 205.26% on the 7th, 14th, 21st, and 28th day. Arsenic poisoned rats treated with morin (As+M) also showed significant response when compared with control animals, but not as drastic as the arsenic-treated rats and the percentage of variation in comparison with control was 64.41% on the 7th day, 47.36% on the 14th day, 94.31% on the 21st day, and 138.44% on the 28th day. Morin-treated control animals (M) showed no significant difference compared to control animals. The percentage of variation compared with control was 1.4% on the 7th day, 5.55% on the 14th day, 344% on the 21st day, and 3.921% on the 28th day.

3.4. Lipid peroxidation

Table 3 shows the LPO levels in different experimental groups during four weeks of study. Control group (C) showed normal LPO levels from 1st week to 4th week of the study. Arsenic-treated group showed significantly higher levels of LPO in comparison with the control group (C), and the percentage of variation in comparison with the control group is as follows: 6.10%, 21.83%, 58.64 %, and 64.97% on 7th, 14th, 21st, and 28th day. Arsenic-poisoned rats treated with morin (As+M) showed significantly lower levels of LPO in comparison to the arsenic-treated animals (As) and the percentage of variation in comparison with the control group is as follows: 3.67% on the 7th day, 8.01% on the 14th day, 29.12% on the 21st day, and 52.55% on the 28th day. Morin-treated control (M) animals showed no variation from control group (percentage of variation in comparison with control group was 0.34% on the 7th day 0.63% on the 14th day, 1.69% on the 21st day and -1.12% on the 28th day).

3.5. Superoxide dismutase

Table 4 shows the activity of SOD in the brains of four experimental groups during the four weeks of the study. SOD activity in the control group showed normal levels from 1st week to 4th week of the study. The arsenic-treated group (As) showed significantly lower levels of SOD from 1st week to 4th week in comparison to the control group (C). The percentage of variation in comparison with the control group is as follows: -46.85%, -41.15%, -49.72%, and -41.63% on the 7th, 14th, 21st, and 28th days. The arsenic- poisoned animals treated with

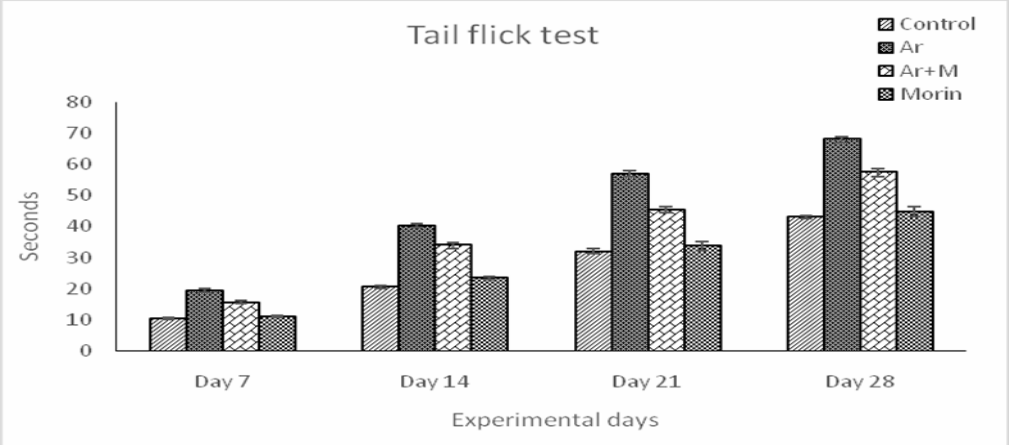

Figure 1. Effect of morin on nociception of arsenic-treated rats.
The values represent Mean ± SE and the percentage of variation between the control and other experimental groups. (All values are significant with $P<0.05$. We expressed the nociception time in seconds).

Table 3. Effect of morin on lipid peroxidation content on brain tissue of arsenic-treated rats.

Experimental Days/Groups	Group-I (Control)	Group-II (As)	Group-III (As+M)	Group-IV (M)	Percentage change over control		
					Group-II	Group-III	Group-IV
Day 7	32.09±0.30	34.05±0.13	33.27±0.23	32.20±0.53	6.10	3.67	0.34
Day 14	49.14±0.41	59.87±1.65	53.08±1.56	49.45±0.14	21.83	8.01	0.63
Day 21	86.04±0.32	136.5±0.39	111.1±0.40	87.5±0.68	58.64	29.125	1.69
Day 28	106.2±0.422	175.2±0.439	162.01±1.710	105±0.33	64.97	52.55	-1.12

The values represent Mean ± SE and percentage of variation between control and other experimental groups. All values are significant with P<0.05. The LPO is expressed in μ MDA/g wt. of tissue.

Table 4. Effect of morin on superoxide dismutase activity (SOD) of arsenic-treated rats.

Experimental Days/Groups	Group-I (Control)	Group-II (As)	Group-III (As+M)	Group-IV (M)	Percentage change over control		
					Group-II	Group-III	Group-IV
Day 7	18.89±0.29	10.04±0.38	15.46±0.83	18.21±0.46	-46.85	-18.15	-3.59
Day 14	26.05±0.42	15.33±0.41	21.60±0.58	24.8±0.58	-41.15	-17.08	-4.79
Day 21	36.12±0.35	18.61±0.93	26.05±0.56	34.23±0.98	-49.72	-27.87	-5.23
Day 28	44.22±0.88	25.81±0.78	33.34±0.89	43.4±0.91	-41.63	-24.60	-1.85

The values represent Mean ± SE and percentage of variation between control and other experimental groups. All values are significant with P<0.05. The SOD activity is expressed in μ SOD/mg of protein.

morin (As+M) showed significant recovery in SOD levels when compared to the arsenic-treated group (As). The percentage of variation in comparison with the control group is as follows: -81.15 % on the 7th day, -17.08% on the 14th day, 27.87% on the 21st day, and -24.60% on the 28th day. Morin-treated control animals group (M) showed no variation in SOD activity in comparison to control group (C) (percentage of variation in comparison with control group was -3.59% on the 7th day -4.79% on the 14th day, -5.23% on the 21st day and -1.85% on the 28th day).

3.6. Catalase

Table 5 depicts the catalase activity of different experimental groups from 1st week to 4th week. The control group (C) showed normal levels of CAT activity in the four weeks of the study. The arsenic-treated group (As) showed a significantly lower level of CAT activity than the control (C) group. The percentage of variation in comparison with the control group is as follows -34.62%, -33.16%, -30.67%, and -32.12% on the 7th, 14th, 21st, and 28th day. The arsenic-poisoned animals treated with morin (As+M) showed significant recovery in comparison with the arsenic-treated group (As) and the percentage of variation in comparison with the control group is as follows: -15.52% on the 7th day, -15.82% on the 14th day, -13.07% on the 21st day, and -15.31% on the 28th day. Morin-treated control animals group (M) showed no variation in comparison with the control group (C) (the percentage of variation in comparison with control group is -2.41% on the 7th day -3.80% on the 14th day, -1.00% on the 21st day and -0.44% on the 28th day).

3.7. Glutathione

Table 6 depicts the results of GSH content in the brain during the four weeks of study. Control group (C) showed regular activity of GSH during the 1st week to 4th week of study. But arsenic-treated group (As) group showed significantly lower levels of GSH content when compared with control (C) and the percentage of variation in comparison with control group is as follows: -35.79%, 28.21%, -30.46 %, and -31.75 % on 7th, 14th, 21st, and 28th days. The arsenic-poisoned animals treated with morin (As+M) showed significant recovery of GSH activity in comparison with the arsenic-treated group (As)

Table 5. Effect of morin on catalase activity of brain tissue of arsenic-treated rats.

Experimental Days/Groups	Group-I (Control)	Group-II (As)	Group-III (As+M)	Group-IV (M)	Percentage change over control		
					Group-II	Group-III	Group-IV
Day 7	19.90±0.99	13.01±0.19	16.81±0.47	19.42±0.23	-34.62	-15.52	-2.41
Day 14	27.86±0.38	18.62±0.24	23.45±0.73	26.8±0.58	-33.16	-15.82	-3.80
Day 21	38.79±0.93	26.89±0.85	33.72±0.45	38.4±0.69	-30.67	-13.07	-1.00
Day 28	54.01±0.77	36.66±0.41	45.74±0.53	53.77±0.67	-32.12	-15.31	-0.44

The values represent Mean ± SE and percentage of variation between control and other experimental groups. All values are significant with P<0.05. The CAT activity is expressed in Units/mg of protein.

Table 6. Effect of morin on the glutathione content of brain tissue of arsenic-treated rats.

Experimental Days/Groups	Group-I (Control)	Group-II (As)	Group-III (As+M)	Group-IV (M)	Percentage change over control		
					Group-II	Group-III	Group-IV
Day 7	22.07±0.67	14.17±0.16	17.48±0.84	20.59±0.83	-35.79	-20.79	-6.70
Day 14	28.03±0.37	20.12±0.37	25.45±0.39	27.01±0.68	-28.21	-9.20	-3.63
Day 21	39.62±0.77	27.55±0.91	34.05±0.50	39.30±0.27	-30.46	-14.05	-0.80
Day 28	55.18±1.23	37.66±1.04	46.57±0.87	54.44±0.96	-31.75	-15.60	-1.34

The values represent Mean ± SE and percentage of variation between control and other experimental groups. All values are significant with P<0.05. The GSH content is expressed in n mole per mg of protein.

and the percentage of variation in comparison with control group is as follows: 7th day by -20.79%, -9.20% on the 14th day, -14.05% on the 21st day, and 15.60% on the 28th day. The Morin-treated control animals group (M) did not show any variation from the control (C) group (the percentage of variation in comparison with the control group is 6.70% on the 7th day -3.63% on the 14th day, -0.80% on 21st day and -1.34% on 28th day).

3.8. Histological studies of brain using Congo red stain

Figure 2 depicts the morphological changes in the midbrain (stained with Congo red) on the seventh day of the experiment (first week). In the normal control group (C), neurons did not show positive staining. In contrast, arsenic-treated group (As) displayed Congo red-positive section, wherein a few neurons had deteriorated myelin in the midbrain. However, a mild Congo red-positive section was observed in the arsenic-poisoned animals treated with morin (As+M) group, wherein a few neurons had deteriorated myelin. Conversely, positive immune stain noticed in myelin sheath bundles in mid-brain was seen in control rats treated with morin (M).

Figure 3 depicts the morphological changes in the hippocampus on the 14th day of experimentation (second week). In the normal control group (Control), foci of Congo red-positive hippocampus neurons and apoptotic hippocampus neurons were observed. In contrast, in the rats given arsenic (arsenic-treated group (As)) Congo red-positive hippocampus neurons and significant degenerated neurons were observed. However, neurons in hippocampus showed less significant positive staining in the arsenic-poisoned animals treated with morin (As+M). Conversely, hippocampus of control rats treated with morin (M) displayed significantly fewer neurons that stained positively with Congo red (red arrow).

Figure 4 depicts the morphological changes in the cerebral cortex (stained with Congo red) on the twenty first day of experimentation (third week). In the normal control group (C), foci of glial cells and neurons in the cerebral cortex did not show a positive reaction with Congo red staining and myelin sheath and nerve bundles showed immune reactivity against Congo red. In the As group (arsenic-treated group) neurons and glial cells in the cerebral cortex did not show positive staining with Congo red and appeared normal. However, myelin sheath and nerve

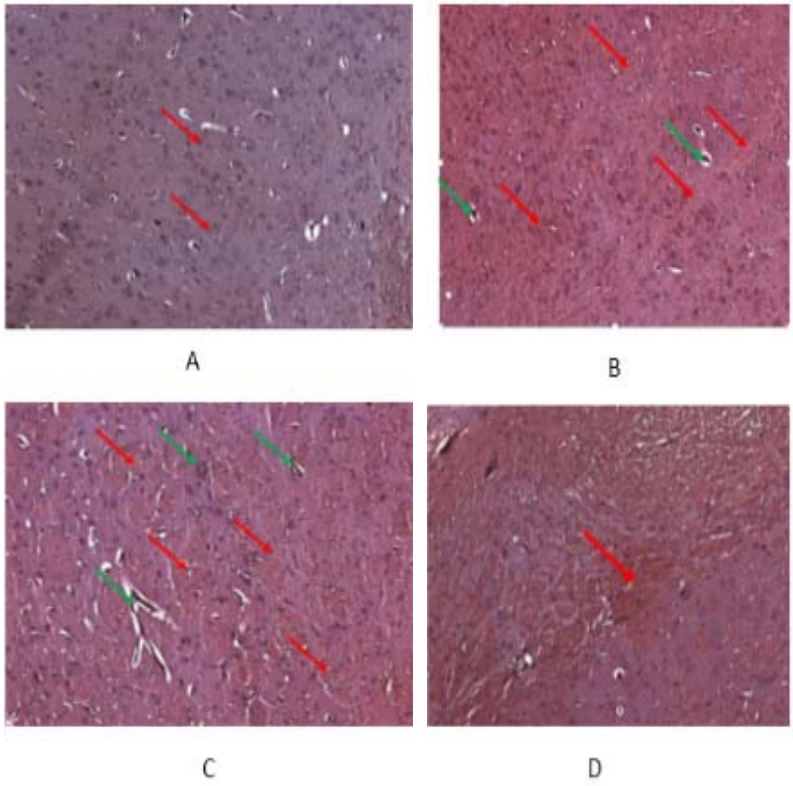

Figure 2

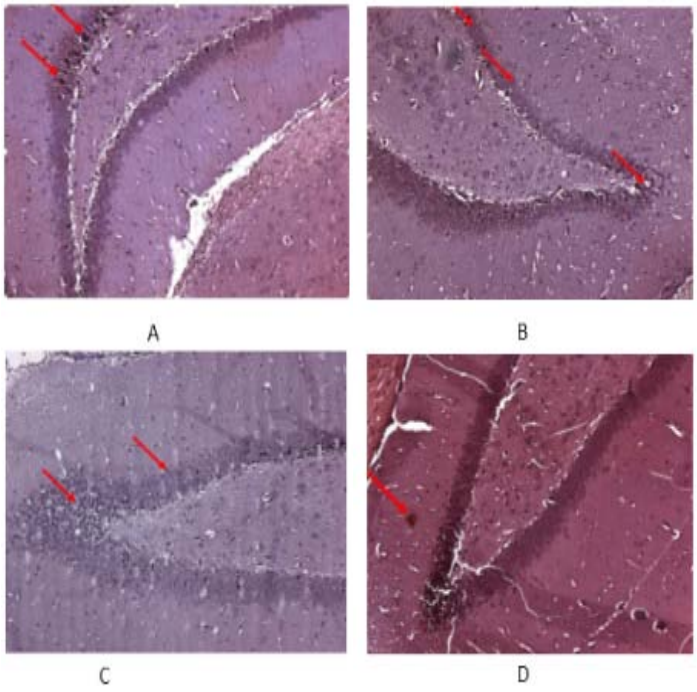

Figure 3

bundles in C. cortex showed positive immune reactivity against Congo red in the arsenic-poisoned animals treated with morin (As+M). Conversely, neurons in the cerebral cortex did not show positive staining in the control rats given morin (M) treatment.

Figure 5 depicts the morphological changes in the cerebral cortex (stained with Congo red) on the twenty eighth day of experimentation (fourth week). In the normal control group (C), neurons in the cerebral cortex did not show any positive staining. In the As group (arsenic-treated group) a few neurons in the cerebral cortex showed positive staining with Congo red. However, neurons in the cerebral cortex showed faint positive staining in the arsenic-poisoned rats treated with morin (As+M). Conversely, neurons in the cerebral cortex did not show positive staining in control rats given morin (M) treatment.

4. DISCUSSION

Many studies on animals have demonstrated the detrimental effects of arsenic on learning, memory and cognitive function, indicating that the brain is a crucial target for arsenic-induced damage [46]. Different brain regions seemed to acquire various inorganic and organic arsenicals [47]. Arsenic quickly passes *via* the blood-brain barrier; it can accumulate in different brain parts and cause various neurological diseases [48]. Brain morphology and physiological alterations are adversely affected by arsenic. According to Breijyeh *et al*., 2020, the brain requires high energy; it is vulnerable to oxidative stress, and arsenic exposure induces oxidative stress damage by reducing antioxidant enzymes in the brain [49]. Baker *et al*., 2018 confirmed that one of the main features of arsenic-induced neurotoxicity is elevated oxidative stress [50]. This research focuses on the oxidative damage to the brain caused by arsenic and the effect of morin therapy.

The analgesic characteristics of morin were evaluated in this study using two significant laboratory tests, the hot plate test and the tail flick test in arsenic-poisoned rats. Many studies have revealed that the hot plate and tail flick test are sensitive to centrally acting toxicants, including arsenic [51]. The tail flick test assesses analgesic effectiveness and delay to thermal pain response. Aguirre-Bauelos *et al*., 2008 [51] demonstrated that no changes in the latency of rat tail withdrawal were induced following a single-dose arsenic injection (acute). But chronic exposure to arsenic (four weeks) induced changes in the latency of tail withdrawal. Baldwin and Cannon (1996) [52] state that the withdrawal reflex in the tail-flick model appears to be modulated by central sensitization, with relative contributions from A-delta and C fibres. Changes in spinal or supraspinal sensitivity to pain processing may result from arsenic exposure. Although prior research has found that acute arsenic exposure did not affect tail flick latency (TFL), we found that persistent exposure for one week changed TFL. And after the fourth week of study, the TFL further escalated. Our findings are consistent with the earlier research reported by Aguirre-Bauelos *et al*. in 2008 [55]. The hot plate test verified whether arsenic could show any

Legend to Figure 2. First week (7^{th} day) of study. Brain (mid-brain) histological sections of all experimental groups stained with Congo red. (**A**) Normal control group (C): Neurons in cerebral cortex did not show positive staining; (**B**) Arsenic-treated experimental group: Congo red-positive section observed in a few degenerated neurons (green arrow) and myelin sheaths in the midbrain (red arrow); (**C**) Arsenic-poisoned rats treated with morin (As+M) group: Congo red-positive section was observed in a few degenerated neurons (green arrow), and an insignificant portion of myelin sheath in the midbrain (red arrow); (**D**) Control animals treated with morin (M) group showed positive immunological staining in myelin sheath bundles in the midbrain (red arrow) (40X, Congo red stained).

Legend to Figure 3. Second week (14^{th} day) of study. Brain (hippocampus) histological sections of all experimental groups stained with Congo red. (**A**) Normal control group (C): foci of Congo red-positive hippocampus neurons and apoptotic hippocampus neurons observed in the hippocampus of the brain (red arrow); (**B**) Arsenic-treated experimental (As) group: Congo red-positive hippocampus neurons and degenerated neurons were observed in the hippocampus of brain (red arrow) (Red colour deposition in the neurons); (**C**) Arsenic-poisoned rats treated with morin (As+M) group: neurons in the hippocampus did show less significant positive staining; (**D**) Control rats treated with morin group (M) shows a few neurons in the hippocampus exhibiting positive staining with Congo red (red arrow) (40X, Congo red stained).

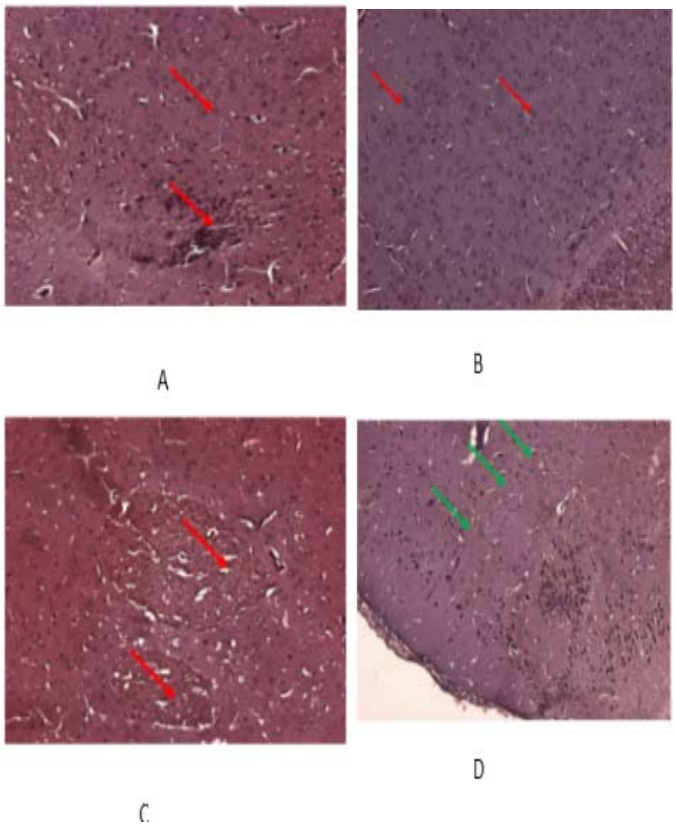

Figure 4

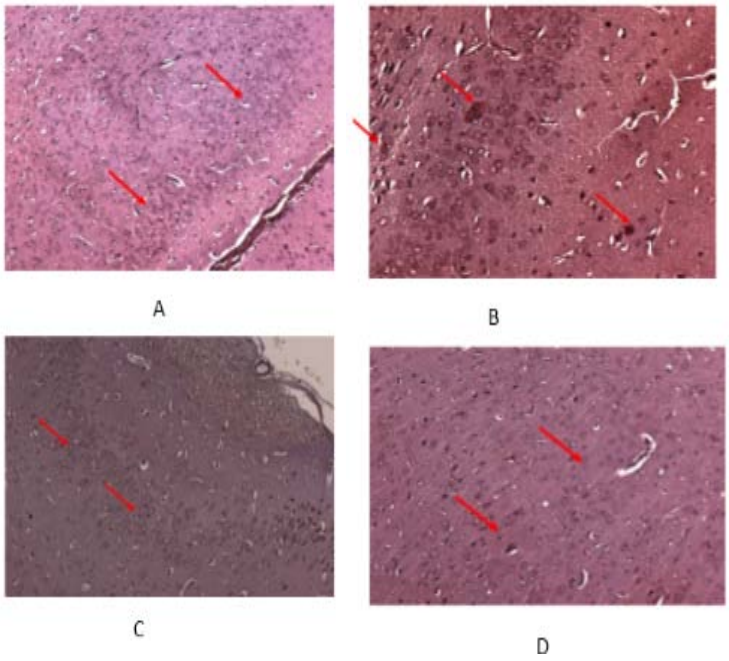

Figure 5

central antinociceptive effect. According to Wani *et al*., 2012 [53], the reaction of mice to unpleasant heat stimuli in the hot plate method (paw licking, jumping) is a supraspinally mediated response. The results of hot plate latencies in rats exposed to arsenic have demonstrated a similar pattern to TFL from the first to the fourth week. The analgesic effect of arsenic in the hot plate test could be because of its interaction with different receptors found in supraspinal areas. The Randal Sellitto pain test, conducted from day 7 to day 28, confirmed the analgesic effect. Morin treatment for 28 days prevented the above mentioned behavioural alterations, demonstrating its neuropharmacological effects.

Arsenic exposure is linked to oxidative damage in the biological system because of the production of free radicals. The current study assessed the oxidative damage caused by Arsenic in brain tissue by evaluating lipid peroxidation, GSH levels, and antioxidant enzymes (SOD, CAT activity) and the significance of morin in reducing arsenic-induced oxidative damage. The protective nature of morin has been well documented [54]. The majority of disease progression needs the effective involvement of reactive oxygen species (ROS) [55], which decreases with morin treatment. Hae-Suk Kim *et al*., 2014 [56] state that controlled actions of morin, which regulate different cell-signalling pathways, are the molecular mechanism underlying such antioxidant capabilities. According to reports, the synthesis of antioxidant enzymes is upregulated in response to excess ROS to protect the cellular ambient environment from free radical activity. According to Hae-Suk Kim *et al*., 2014 [56], the expression of antioxidant proteins such as catalase, glutathione reductase, superoxide dismutase, and glutathione peroxidase is stimulated by morin. In the present study, the LPO level in rats treated with arsenic increased from the first to the fourth week; in rats treated with morin, this elevation was reduced. According to Choudhury *et al*., 2017 [57], the presence of a double bond at the C2-C3 position and a hydroxyl group at the C3 position in the chemical structure of morin is critical for the reduction of LPO. But hydroxyl groups (ring B) at 2′ and 4′ locations also contribute significantly to the decrease in lipid peroxidation. In the brains of arsenic-treated rats, GSH (reduced glutathione) levels were much lower, whereas morin-treated animals had significantly increased GSH levels. Catalase and superoxide dismutase activity has also demonstrated a similar trend of decreased activity in animals treated with arsenic; however, the activity of both enzymes has been rescued by morin treatment. These findings are consistent with other investigations documenting antioxidant action of morin in various tissues under various clinical conditions [58, 59, 60].

The increased oxidative stress in the brain after arsenic exposure can seen in the histological observations in the mid-brain during the first week of the experiment, the hippocampus during the second week, and the cerebral cortex during the third and fourth weeks, which were stained with Congo red. The midbrain of rats treated with arsenic exhibited a segment of deteriorated neurons, and myelin sheaths were positive for Congo red. The

Legend to Figure 4. Third (21st day) week of study. Brain (cerebral cortex) histological sections of all experimental groups stained with Congo red. (**A**) Normal control group (C): foci of glial cells and neurons in the cerebral cortex did not show a positive reaction with Congo red staining and Myelin sheath and nerve bundles showed immune reactivity against Congo red (red arrow); (**B**) Arsenic-treated experimental (As) group: neurons and glial cells in cerebral cortex did not show positive staining with Congo red and appeared normal – red arrow; (**C**) Arsenic-poisoned rats treated with morin (As+M) group: myelin sheath and nerve bundles in the cerebral cortex showed positive immune reactivity against Congo red (red arrow); (**D**) Control animals treated with morin (M) did not show positive staining in cerebral cortex for neurons (green arrow) (40X, Congo red stained).

Legend to Figure 5. Fourth (28th day) week of study. Brain (cerebral cortex) histological sections of all experimental groups stained with Congo red. (**A**) Normal control group (C): neurons in cerebral cortex did not show any positive staining; (**B**) Arsenic-treated experimental (As) group: a few neurons in cerebral cortex showed positive staining with Congo red (red arrow); (**C**) Arsenic-poisoned rats treated with morin (As+M) group: neurons in the cerebral cortex showed faint positive staining; (**D**) Control animals treated with morin (M) did not show positive staining in cerebral cortex for neurons (red arrow) (40X, Congo red stained).

hippocampus of rats treated with arsenic showed Congo red-positive hippocampus neurons, degenerated neurons, and deposition in the neurons. The cerebral cortex showed a positive Congo red stain for neuronal degeneration. On the other hand, morin treatment in arsenic rat's showed a significant improvement in the overall histo-architecture of brain regions such as the midbrain, cerebral cortex, and hippocampus. ROS is associated with nervous system damage caused by arsenic-induced oxidative stress [61]. On the other hand, morin treatment significantly reduced neurotoxicity, and the treatment's positive impact could be attributed to its antioxidant properties.

The observed alterations in nociceptive responses could mainly be explained by fluctuations in antioxidant molecules in the brain, particularly GSH in arsenic-toxic rats. GSH is the most prevalent antioxidant molecule essential for scavenging free radicals, inhibiting lipid peroxidation, and shielding the brain from oxidative stress. In arsenic-toxic rats, oxidative stress can mediate neurodegeneration in the cerebral cortex [62], causing nociceptive alterations in the hippocampus and bringing about behavioural impairments [63]. Alongside these results, we also found that when arsenic-toxic rats were treated with morin, there was a decrease in the total GSH, SOD and CAT antioxidant enzymes in the brain. Our findings confirm the antioxidant activity of morin.

5. CONCLUSION

In conclusion, the current findings indicate that morin can effectively mitigate the neurotoxicity caused by arsenic. Morin could reduce oxidative stress by boosting the endogenous antioxidant system, and improving arsenic-induced neurotoxicity impairment. Thus, our findings show that the antioxidant mechanism of morin against arsenic neurotoxicity can help in preventing pain and inflammation caused by arsenic.

AUTHOR CONTRIBUTIONS

The first and second author did this work under the supervision of the third author.

CONFLICT OF INTEREST STATEMENT

No conflict of interest associated with this work.

REFERENCES

1. Singh A. K. 2006, Curr. Sci., 91, 599–606.
2. Shi, H., Shi, X. and Liu, K. J. 2004, Mol. Cell Biochem., 255, 67-78.
3. Kapaj, S., Peterson, H., Liber, K. and Bhattacharya, P. 2006, J. Environ. Sci. Health A Tox. Hazard Subst. Environ. Eng., 41, 2399–2428.
4. Mazumder, D. N. G. 2008, Indian J. Med. Res., 128, 436–447.
5. Ratnaike, R. N. 2003, Postgrad Med. J., 79, 391–396.
6. Bhattacharya, S. and Haldar, P. K. 2013a, Pharm. Biol., 51, 1477–1479.
7. Rodriguez, V. M., Jimenez-Capdeville, M. E. and Giordano, M. 2003, Toxicol. Lett., 145(1), 1–18.
8. Jomova, K., Jenisova, Z., Feszterova, M., Baros, S., Liska, J., Hudecova, D., Rhodes, C. J. and Valko, M. 2011, J. Appl. Toxicol., 31(2), 95–107.
9. Rai, A., Tripathi, P., Dwivedi, S., Dubey, S., Shri, M., Kumar, S., Tripathi, P. K., Dave, R., Kumar, A., Singh, R., Adhikari, B., Bag, M., Tripathi, R. D., Trivedi, P. K., Chakrabarty, D. and Tuli, R. 2010, Chemosphere., 82(7), 986–995.
10. Aguirre-Bañuelos, P., Escudero-Lourdes, C., Sanchez-Peña, L. C., Del Razo, L. M. and Perez-Urizar, J. 2008, Toxicol. Appl. Pharmacol., 229(3), 374-85.
11. Gimbrone Jr., M. A., Topper, J. N., Nagel, T., Anderson, K. R. and Garcia-Cardenas, G. 2000, Ann. NY. Acad. Sci., 902, 230–239.
12. Burian, M. and Geisslinger, G. 2005, Pharmacol. Ther., 107,139–154.
13. Kumar, A, Kumar, R, Rahman, M. S., Iqubal, M. A., Anand, G., Niraj, P. K. and Ali, M. 2015a, Avicenna J. Phytomed., 5, 355–364.
14. Dua, T. K., Dewanjee, S., Gangopadhyay, G., Khanra, R., Zia-Ul-Haq, M. and Feo, V. D. 2015, J. Translat Med., 13, 81.
15. Das, A. K., Dewanjee, S., Sahu, R., Dua, T. K., Gangopadhyay, M. and Sinha, M. K. 2010c, Environ Toxicol. Pharmacol., 29, 64–69.
16. Sarkozi, K., Papp, A., Mate, Z., Horvath, E., Paulik, E. and Szabo, A. 2015, Acta Biol. Hung., 66, 14–26.

17. Jahan, S., Iftikhar, N., Ullah, H., Rukh, G. and Hussain, I. 2015, Syst. Biol. Reprod. Med., 61, 89–95.
18. Yousef, M. I., El-Demerdash, F. M. and Radwan, F. M. E. 2008, Food Chem. Toxicol., 46, 3506–3511.
19. Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A. H. and Jaremko, M. 2020, Molecules, 25.
20. Chen, C. Olive, Y. and Blumberg, J. B. 2008, Journal of Clinical Nutrition., 17(1), 329-32.
21. Jiminez-Escrig, A., Mariela Rincon, Raquel Pulido. and Fulgencio Saura-Calixto. 2001, Journal of Agricultural and Food Chemistry, 49(11), 5489-93.
22. Lamounier, K. C, Cuhan, L. C. S., Morais, S., Franciso Jose Torres Aquino, Chang, R., do Nascimento, E. A., de Souza, M. G. M., Martins, C. H. G. and Cunha, W. R. 2012, Evidence-Based Complementary and Alternative Medicine : eCAM., 2012, 451039.
23. Tsao, R., Raymond Yang. and Christopher Young, J. 2003, Journal of Agricultural and Food Chemistry, 51(22), 6445-51.
24. Hussain, J., Ali, L., Khan, A. L., Rehman, N. U., Jabeen, F., Kim, J.-S. and Al-Harrasi, A. 2014, Molecules, 19, 17763-17772.
25. Osman, A. and Makris, D. P. 2011, International Food Research Journal, 18(3), 1039-43.
26. Trichopoulou, A., Vasilopoulou, E., Hollman, P., Chamalides, Ch., Foufa, E., Kaloudis, Tr., Kromhout, D., Miskaki, Ph., Petrochilou, I., Poulima, E. and Stafilakis, K. Theophilou, D. 2000, Food Chemistry., 70(3), 319-23.
27. Weiduo Si, Joshua Gong, Rong Tsao, Milosh Kalab, Raymond Yang. and Yulong Yin. 2006, Journal of Chromatography A., 1125(2), 204-10.
28. Whitehead, T. P, David Robinson, Sharon Allaway, Jacquie Syms, and Ann Hale. 1995, Clinical Chemistry, 41(1), 32-35.
29. Somoza, VLindenmeier M., Wenzel, E., Frank, O., Erbersdobler, H. F. and Hofmann, T. 2003, J Agric Food Chem., 51(23), 6861-9.
30. Decker, E. 2002, Cereal Foods World, 47(8), 370-73.
31. Lemkul, J. A and Bevan, D. R. 2010, Biochemistry, 49(180), 3935-46.
32. Caselli, A. Paolo Cirri, Santi Alice. and Paolo Paoli. 2016, Current Medicinal Chemistry., 23(8), 774-91.
33. Gottlieb, M. 2006, Neurobiology of Disease, 23(2), 374-86.
34. Paoli, P. 2013, Biochimica et Biophysica Acta - General Subjects., 1830(4), 3102-11.
35. Zhang, S., Yang, X. and Morris, M. E. 2004, Molecular Pharmacology, 65(5), 1208-16.
36. Wu, T. W. and Ling, H. Z. 1994, Biochemical Pharmacology, 47(6), 1099-103.
37. Prahalathan, P., Kumar, S. and Raja, B. 2012, Asian Pacific Journal of Tropical Biomedicine, 2(6), 443-48.
38. Richard, E. Chipkin, Miklos B. Latranyi, Louis C. Iorio. and Allen Barnett. 1983, Journal of Pharmacological Methods., 10(3), 223-229.
39. Lavich, T. R., Cordeiro, R. S. B., Silva, Patrícia, Martins and Marco. 2005, Brazilian journal of medical and biological., 38, 445-51.
40. D'Amour, F. E. and Smith, D. L. 1941, J. Pharmacol. Exp Ther., 72(1), 74-9.
41. Wills, E. D. 1966, Biochem. J., 99(3), 667-76.
42. Kono, Y. 1978, Arch Biochem Biophys., 186(1), 189-95.
43. Luck, H. 1971, Catalase. In: Hu, B., Ed., Methods of Enzymatic Analysis, 3, 279.
44. Irfan Rahman , Aruna Kode, Saibal K. Biswas, 2006, Nat Protoc., 1(6), 3159-65
45. Luna, L. G. 1968, Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd Edition, McGraw-Hill, New York.
46. Lewchalermvong, K., Rangkadilok, N., Nookabkaew, S., Suriyo, T. and Satayavivad, J. 2018, J. Agric. Food Chem., 66, 3199–3209.
47. Chen, P., Miah, M. R. and Aschner, M. 2016, F1000 Research., 5, 366
48. Uttara, B., Singh, A., Zamboni, P. and Mahajan, R. 2009, Curr. Neuropharmacol, 7, 65–74.
49. Breijyeh, Z. and Karaman, R. 2020, Molecules, 25, 5789.

50. Baker, B. A., Cassano, V. A. and Murray, C. J. 2018, Occup. Environ. Med., 60, e634–e639.
51. Aguirre-Bañuelos, P., Escudero-Lourdes, C., Sanchez-Peña, L. C., Del Razo, L. M. and Perez-Urizar, J. 2008, Toxicol. Appl. Pharmacol., 229(3), 374-85.
52. Baldwin, A. E. and Cannon, J. T. 1996, Pain., 67, 163–172.
53. Wani, T. A., Kumar, D. and Prasad, R. 2012, Indian J. Pharmacol., 44(4), 493–499.
54. Venkata Krishna Rao Balaga, Aditi Pradhan, Riya Thapa, Neeraj Patel, Riya Mishra and Neelam Singla. 2023, Modern Chinese Medicine., 7, 100264.
55. Sies, H. 1986, Angewandte Chemie International Edition in English., 25(12), 1058-71.
56. Hae-Suk Kim, Michael J. Quon. and Jeong-a Kim. 2014, Redox Biology., 2, 187-195.
57. Amarendranath Choudhury, Indrajeet Chakraborty, Tuhin Subhra Banerjee, Dhilleswara Rao Vana and Dattatreya Adapa. 2017, Int. J. Med Re & Health Sciences., 6(11), 175-194.
58. Subash, S. and Subramanian, P. 2009, Molecular and Cellular Biochemistry., 327(1-2), 153-61.
59. Kim Ji, M., Lee, E. K., Park, G., Kim, M. K., Yokozawa, T., Yu, B. P. and Chung, H. Y. 2010, Free Radical Research., 44(4), 454-61.
60. Yager, J. Y., Brucklacher, R. M. and Vannucci, R. C., 1991, American Journal of Physiology, 261(4), H1102-8.
61. Anupama Sharma, Chaoba Kshetrimayum, Harsiddha G. Sadhu. and Sunil Kumar. 2018, Environmental Science and Pollution Research., 25, 23946–23953.
62. Hosseinzadeh, A., Houshmand, G., Kalantar, M. and Hamidreza, K. 2020, Comp. Clin. Pathol., 29, 621–629.
63. Selvakumar K., Bavithra, S., Ganesh, L., Krishnamoorthy, G., Venkataraman, P. and Arunakaran, J. 2013, Toxicol Lett., 222, 45–54.