International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.1479.1487

Research Article
Luteolin Ameliorating Effects of Endoplasmic Reticulum Stress and Mitochondrial Dysfunction in an ASD Rodent Model

[1]Abir Ben Bacha, [1]Mona Alonazi, [1]Enas Muhammad Aljehani, [1]Altaf N. Alabdali, [1]Ramesa Bhat, [2]Mohammed Almuayrifi, [3]Habib Horchani and [4]Afaf El-Ansary

[1]Department of Biochemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia
[2]Experimental Surgery and Animal Laboratory, College of Medicine, King Saud University, Riyadh 11495, Saudi Arabia
[3]Department of Science, College of Rivière-Du-Loup, Rivière-Du-Loup, QC G5R 1R1, Canada
[4]Autism Center, Lotus Holistic Alternative Medical Center, P.O. Box 110281, Abu Dhabi, United Arab Emirates

Abstract

Background and Objective: Luteolin, the most powerful and pleiotropic flavonoids significantly reverses the effects of oxidative stress and cognitive impairment. This study aimed to investigate its protective and therapeutic effects on brain intoxication induced by propionic acid (PPA) in male albino rats. **Materials and Methods:** Fifty neonates (~21 days old, 80-100 g) male albino rats were randomly assigned to five groups: Control, the PPA-treated, luteolin-treated, the protective and the therapeutic groups. A panel of antioxidants, endoplasmic reticulum (ER) stress and mitochondrial dysfunction markers were measured using Enzyme-Linked Immunosorbent Assay (ELISA) kits in brain homogenates of all tested groups. **Results:** The data demonstrated luteolin's therapeutic and protective efficacy by improving specific biochemical indicators compared to the PPA-treated and control groups. The PPA treatment showed an increase in oxidative stress markers such as lipid peroxidation, coupled with increased catalase activity and glutathione-S-transferase with a decrease in non-enzymatic antioxidants such as glutathione and vitamin C. Furthermore, caspase-3 level decreased as a mitochondrial dysfunction biomarker, whereas glucose-regulated protein 78 level, as an ER stress marker did not demonstrate a significant difference compared to PPA-treated or control groups. **Conclusion:** Current findings suggest that PPA caused neurotoxicity-associated oxidative stress while luteolin was neuroprotective and neurotherapeutic, making it a potential candidate for ER therapy as an etiology of neurological illnesses among which is autism spectrum disorders.

Key words: Autism spectrum disorders, endoplasmic reticulum stress, mitochondrial dysfunction, oxidative stress, propionic acid

Citation: Ben Bacha, A., M. Alonazi, E.M. Aljehani, A.N. Alabdali and R. Bhat *et al*., 2024. Luteolin ameliorating effects of endoplasmic reticulum stress and mitochondrial dysfunction in an ASD rodent model. Int. J. Pharmacol., 20: 1479-1487.

Corresponding Author: Abir Ben Bacha, Department of Biochemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia
Tel: +00966-11-8050129

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Clinically, Autism Spectrum Disorder (ASD) is a neurodevelopmental illness characterized by decreased social interaction and communication skills as well as repetitive behaviors[1]. The wide range of ways in which people exhibit autistic traits in their clinical presentations is the reason these impairments are referred to as a spectrum. The majority of persons with ASD are categorized as either having average to higher intellectual capacity or having low linguistic and intellectual talent[2]. While there is no known treatment for ASD, early diagnosis raises awareness of pathologic behaviors linked with the disorder before they worsen. Early modification of the essential characteristics of autism can reduce the rate at which symptoms worsen and optimize the benefits of early intervention[3]. Environmental contaminants have been proposed as possible causes of learning and emotional disturbances at a young age and neurodegenerative diseases in later life[4]. In this context, propionic acid (PPA) has been shown to frequently cause a number of behavioral alterations and neuroinflammatory reactions in rats that are evocative of ASD. This dietary short-chain fatty acid is produced by enteric bacteria in the stomach and is frequently used as a food preservative. Among its many beneficial properties include neuroendocrine modulation, tumor suppression and anti-inflammatory properties. Additionally, PPA serves as the primary mediator between the brain and the gut microbiota through bidirectional communication of the gut microbiota-brain axis[5,6].

On the other hand, high PPA could have neurotoxic effects that are linked to autism, including immune system malfunction, metabolic disorders and mitochondrial oxidative stress[5,7]. The validity of the PPA-induced rodent model of autism is supported by the recent work of Abuaish *et al.*[5], which showed that PPA administration-induced autism-like neurobehaviors such as reduced probing activity, increased aggressive behavior and impaired social interaction through the induction of abnormal neural cell organization. Notably, records indicate that PPA plays a major role in altering the modeling of human fetal-derived neural stem cells, which results in gliosis, defective neurocircuitry and inflammatory response, all of which are observed in people with ASD[8]. Additionally, PPA easily crosses the gut-brain barrier and modifies brain networks that are involved in neurotransmission, neuroinflammation and energy metabolism[9-11]. Remarkably, it was demonstrated that a neurotoxic dose of PPA given orally to mice altered their social abilities and cognitive flexibility in addition to causing other changes resembling those seen in autistic people[9,11]. It is also worth noting that in some animal models of ASD, endoplasmic reticulum (ER) stress increases. This is indicated by increased expression of synaptic factors, suppression of neurite growth and stimulation of the unfolded protein response[12]. Both Glucose-Regulated Protein-78 (GRP78) and Ubiquitin-Protein Ligase (HRD1) are increased during ER stress and are thought to be markers for ER stress in both tissue and blood[12,13]. Nowadays, the development of neuroprotective drugs derived from natural sources has received a lot of interest since neurological diseases are currently one of the most significant and difficult health issues in the world. Numerous *in vitro* and *in vivo* investigations have shown these phytochemicals to have great efficacy and few side effects. Dietary flavonoids, which are present in a variety of fruits and vegetables, are a significant and widespread class of phytochemicals that are bioactive compounds[14]. Broccoli, pepper, thyme and celery are among the plant products that contain luteolin, a significant flavonoid. Several investigations have demonstrated the positive neuroprotective benefits of luteolin, such as anti-oxidant free radical scavenging and anti-inflammatory potency, both *in vivo* and *in vitro*[14-18].

Based on the fact that mitochondrial dysfunction and ER stress are common features of neurological disorders, mitotherapeutix may be a valuable approach that could help in the treatment of multiple neurodegenerative and neurodevelopmental disorders among which is ASD. The purpose of this study was to examine the possible therapeutic and preventive effects of luteolin as a modulator of specific biochemical markers that indicate endoplasmic reticulum stress, mitochondrial malfunction and oxidative stress as neurotoxic effects of PPA.

MATERIALS AND METHODS

Study area: The study was performed in the period from July, 2023 to May, 2024 at King Saud University, College of Science, Department of Biochemistry, Riyadh, Kingdom of Saudi Arabia.

Animals: A total of 50 young male albino rats weighing about 80-100 g were obtained from the Experimental Surgery and Animal Laboratory (College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia) and kept in a cage ($40 \times 35 \times 20$ cm^3) with a controlled temperature of 21 ± 1 °C and light conditions (light on at 9:00 and light off at 21:00). Rats had free access to food (standard laboratory animal feed pellets) and water. Animals were randomly divided into five groups, each consisting of 10 animals: Group I (control group): Rats received only phosphate-buffered saline;

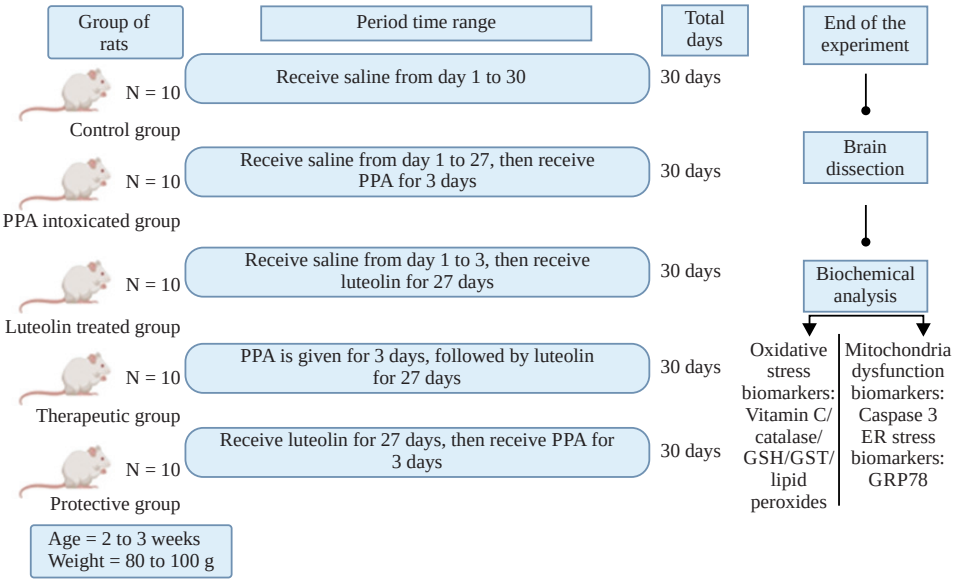

Fig. 1: Diagrammatic scheme of the animal experiments

ER: Endoplasmic reticulum, GRP78: Glucose-Regulated Protein-78, GSH: Glutathione, GST: Glutathione-S-transferase and PPA: Propionic acid

Group II (PPA-intoxicated group) rats received PPA at a dose of 250 mg/kg b.wt., for 3 days; Group III (luteolin-treated group) rats received luteolin at a dose of 50 mg/kg b.wt./day for 27 days; Group IV (therapeutic group): Rats received a neurotoxic dose of 250 mg/kg b.wt./day of PPA for 3 days followed by luteolin at the same dose of 50 mg/kg b.wt./day for 27 days; Group V (protective group): Rats received luteolin at the same dose of 50 mg/kg b.wt./day for 27 days followed by a neurotoxic dose of PPA (250 mg/kg b.wt./day) for 3 days (Fig. 1).

At the end of the experiment (day 30), the brains separated from the sacrificed animals were washed and sliced into fragments (~1 g). Using a Teflon homogenizer (Merck KGaA, Darmstadt, Germany), 1 g of each brain tissue was homogenized for 3 min in 10 mL of double-distal water. Supernatants, thus obtained, were kept at 80°C for further analysis.

The experimental protocol was approved via the Ethics Committee for Animal Research at King Saud University, Riyadh, KSU-SE-23-26.

Biochemical analysis: Caspase-3 and GRP78 levels were investigated in all brain homogenates using ELISA kits, products of MyBioSource (San Diego, USA) following the manufacturers' instructions. Catalase and glutathione-S-transferase (GST) activities along with Glutathione (GSH), vitamin C and lipid peroxides levels were measured by spectrophotometry according to the methods of Researchers[19-23], respectively. All measurements were carried out in triplicate and the mean of 3 separate readings was determined.

Statistical analysis: All data were carried out by one-way ANOVA followed by Tukey's multiple comparison tests. Only $p \leq 0.05$ were considered significant. Results illustrated as Mean ± Standard Error of the mean (SEM) were obtained using GraphPad Prism (version 9.5.0).

RESULTS

The data of this study are expressed as the Mean ± SEM of all biochemically measured parameters in the five studied groups. Table 1 demonstrated the brain homogenate levels of GSH, lipid peroxidation, catalase, GST, vitamin C, caspase-3 and GRP78, in addition to their percentage change relative to control for all the tested groups. The therapeutic-treated group exhibited an increase in GST (240.33%), GSH (122.35%) and catalase (+153.96%), with a concomitant decrease in vitamin C (87.13%) in comparison with the control. Table 1 also showed that luteolin is an antioxidant that lowers lipid peroxidation in two groups compared to the control group: The luteolin group with recorded values of (87.49%) that was treated with luteolin only and the protective group that was treated with PPA and then treated with luteolin with recorded values of 96.52%. The therapeutic group that was treated with luteolin after PPA treatment, with recorded values of

Table 1: Level of measured markers in brain homogenate of treated rats compared to control group

Parameter	Groups	Mean±SD	Mean±SEM	Change (%)	p-value[b]	p-value[a]
GSH (µg/g of brain tissue)	Control	115.65±29.92	12.21	100.00	-	0.0006
	PPA	61.65±19.54	6.182	53.30	0.0006	
	Luteolin	113.25±23.46	7.422	97.92	0.8605	
	Therapeutic	141.05±78.86	24.94	122.35	0.6185	
	Protective	160.81±34.85	12.32	139.05	0.0258	
GST (IU/g of brain tissue)	Control	24.27±12.59	5.142	100.00	-	0.0116
	PPA	30.26±12.13	4.044	124.69	0.3770	
	Luteolin	45.88±25.46	8.054	189.06	0.0750	
	Therapeutic	58.32±22.57	7.140	240.33	0.0046	
	Protective	45.55±22.23	7.862	187.70	0.0583	
Lipid peroxidation (µmol/g of brain tissue)	Control	51.03±18.60	7.594	100.00	-	0.2930
	PPA	61.7±30.99	9.802	120.09	0.4576	
	Luteolin	44.65±7.86	2.487	87.49	0.3496	
	Therapeutic	56.86±14.25	4.509	111.41	0.4910	
	Protective	49.26±8.75	3.095	96.52	0.8151	
Vitamin C (µg/g of brain tissue)	Control	172.15±250.91	102.4	100.00	-	0.0376
	PPA	147.13±86.89	27.48	85.46	0.7738	
	Luteolin	355.72±241.88	76.49	206.63	0.1691	
	Therapeutic	150.01±106.28	33.61	87.13	0.8073	
	Protective	152.85±110.39	39.03	88.78	0.8481	
Catalase (IU/g of brain tissue)	Control	0.61±0.186	0.07632	100.00	-	0.4712
	PPA	0.9570±0.4718	0.1650	161.00	0.1075	
	Luteolin	0.86±0.66	0.2092	141.50	0.3808	
	Therapeutic	0.94±0.65	0.2083	153.96	0.2570	
	Protective	0.58±0.43	0.1551	96.22	0.9122	
Caspase-3 (ng/g of brain tissue)	Control	31.84±31.84	1.935	100.00	-	0.0017
	PPA	20.72±6.900	2.182	65.07	0.0001	
	Luteolin	26.25±8.342	2.638	82.44	0.1224	
	Therapeutic	33.12±21.92	6.932	104.02	0.874	
	Protective	12.10±6.729	2.128	38.00	0.0001	
GRP78 (ng/g of brain tissue)	Control	138.3±70.80	25.03	100.00	-	0.0128
	PPA	130.4±32.36	10.23	94.28	0.7590	
	Luteolin	92.12±32.06	10.14	66.60	0.0832	
	Therapeutic	130.1±30.50	9.644	94.07	0.7455	
	Protective	76.40±51.48	16.28	55.24	0.047	

[a]p-value between all groups using One-way ANOVA, [b]p-value between the control group and other groups using the unpaired t-test, two-tailed p-value, GRP78: Glucose-Regulated Protein-78, GSH: Glutathione, GST: Glutathione-S-transferase and PPA: Propionic acid

+111.41%, also decreased lipid peroxidation but still increased values compared to the control group. Likewise, the protective-treated group exhibited a significant increase in GSH (+139.05%), GST (+187.70%) and a drastic decrease in caspase-3 (38.00%), GRP78 (55.24%) and vitamin C (88.78%) in comparison with the control group. The brain homogenates of protective and therapeutic-treated animals also showed improvement in all the tested parameters, as shown in Table 1. In addition, individual values represent the mean and standard error of the mean, with a significant p-value at *0.05, **0.01, ***0.001 and ****0.0001 of all parameters measured in the brain homogenate of the treated rats presented in Table 1 compared to the control group. The ROC curve analysis, presented in Table 2 shows AUC, specificity and sensitivity. Finally, Pearson correlation between the different measured parameters was performed and presented in Table 3, showing either a positive or negative correlation.

DISCUSSION

The current study's findings showed that luteolin has therapeutic and preventive benefits against the neurotoxic effects of PPA in young male albino rats, which serve as a rodent model for ASD. This confirmed luteolin's antioxidant and anti-ER stress properties, as seen by the improvement of GSH, GST, catalase and lipid peroxides as measures of oxidative stress and GRP78 as a measure of ER stress, with no effect on caspase-3, a pro-apoptotic marker. Given that ER stress and oxidative stress might be linked to a variety of molecular cascade responses, including mitochondrial dysfunction, neuroinflammation and cell death, all of which contribute to the etiology of ASD as a neurodevelopmental disorder, using luteolin as a nutritional intervention may be critical. The finding could find support in multiple recent studies that prove that luteolin is one of the most powerful

Table 2: ROC analysis of the parameters measured in the brain homogenate of the treated rats

Parameter	Groups	AUC	Likelihood ratio	Sensitivity (%)	Specificity (%)	p-value
GSH (µg/g of brain tissue)	PPA	0.9333	1.200	100.0	16.67	0.0048
	Luteolin	0.5583	0.6000	10.00	83.33	0.7042
	Therapeutic	0.5250	0.8400	70.00	16.67	0.8708
	Protective	0.8125	1.125	75.00	33.33	0.0528
GST (IU/g of brain tissue)	PPA	0.6417	1.350	90.00	33.33	0.3566
	Luteolin	0.7583	3.600	60.00	83.33	0.0927
	Therapeutic	0.9250	5.400	90.00	83.33	0.0057
	Protective	0.8021	1.750	87.50	50.00	0.0612
Lipid peroxidation (µmol/g of brain tissue)	PPA	0.6500	0.9600	80.00	16.67	0.3290
	Luteolin	0.6500	0.6000	10.00	83.33	0.3290
	Therapeutic	0.6500	1.400	70.00	50.00	0.3290
	Protective	0.5208	0.9000	75.00	16.67	0.8973
Vitamin C (µg/g of brain tissue)	PPA	0.6750	1.200	100.0	16.67	0.2548
	Luteolin	0.7500	0.6000	10.00	83.33	0.1037
	Therapeutic	0.6167	2.100	70.00	66.67	0.4477
	Protective	0.6250	1.125	75.00	33.33	0.4386
Catalase (IU/g of brain tissue)	PPA	0.7407	0.9333	77.78	16.67	0.1255
	Luteolin	0.5917	2.400	40.00	83.33	0.5508
	Therapeutic	0.6417	1.400	70.00	50.00	0.3566
	Protective	0.6042	1.250	62.50	50.00	0.5186
Caspase-3 (ng/g of brain tissue)	PPA	0.8813	1.143	100.0	12.50	0.0067
	Luteolin	0.7063	4.000	50.00	87.50	0.1426
	Therapeutic	0.6188	1.600	60.00	62.50	0.3986
	Protective	0.9938	2.000	100.0	50.00	0.0004
GRP78 (ng/g of brain tissue)	PPA	0.5750	1.143	100.0	12.50	0.5940
	Luteolin	0.7625	1.600	20.00	87.50	0.0621
	Therapeutic	0.6000	1.867	70.00	62.50	0.4772
	Protective	0.7625	2.400	60.00	75.00	0.0621

AUC: Area under the curve, GRP78: Glucose-Regulated Protein-78, GSH: Glutathione, GST: Glutathione-S-transferase and PPA: Propionic acid

Table 3: Pearson's correlations between the measured parameters

Parameter	R (person correlation)	p-value	
Catalase (IU/g of brain tissue) with lipid peroxidation (mmol/g of brain tissue)	-0.3195	0.0345*	N[b]
GST (IU/g of brain tissue) with GSH (µg/g of brain tissue)	0.3513	0.0194*	P[a]
Lipid peroxidation (mmol/g of brain tissue) with catalase (IU/g of brain tissue)	-0.3195	0.0345*	N[b]
Lipid peroxidation (mmol/g of brain tissue) with GRP78 (ng/g of brain tissue)	0.4942	0.0007***	P[a]
GSH (µg/g of brain tissue) with GST (IU/g of brain tissue)	0.3513	0.0194*	P[a]
GRP78 (ng/g of brain tissue) with lipid peroxidation (mmol/g of brain tissue)	0.4942	0.0007***	P[a]

***Correlation is significant at the 0.001 level, *Correlation is significant at the 0.05 level, [a]Positive correlation, [b]Negative correlation, GRP78: Glucose-Regulated Protein-78, GSH: Glutathione, GST: Glutathione-S-transferase and PPA: Propionic acid

and pleiotropic flavonoids, acting as a protective antioxidant and anti-inflammatory throughout the body by altering critical regulatory molecules[24,25]. Indeed, one of the strategies commonly used for assessing the effect of luteolin on the functioning of the brain in an ASD rat model is using an oral neurotoxic dose of PPA (250 mg/kg b.wt./day) for 3 days[16,26].

Table 1 shows considerable GSH depletion in the PPA-treated group compared to the control ($p<0.0006$). This was supported by the findings of MacFabe[11] and El-Ansary *et al.*[26], who found that a neurotoxic dose of PPA increased oxidative stress indicators, decreased antioxidant synthesis and impaired GSH metabolism in most brain areas. Furthermore, it has been well-established that autism is associated with increased oxidative damage[27,28]. Table 1 also demonstrates that in these animals, the luteolin effect was remarkably effective in restoring GSH levels in the brain, with values of +22.35% for the therapeutic group and +139.05% for the protective group ($p<0.0258$).

The recorded effect of luteolin in restoring the brain GSH level can be supported by the work of Mohammad *et al.*[6] and Alsubaiei *et al.*[29], who reported that consumption of artichoke as luteolin-rich food showed a significant increase of GSH in PPA-treated rats as rodent models of autism. This can be explained, interestingly, by the fact that luteolin increases reduced GSH levels and GSH synthetase expression, the latter of which catalyzes the second step of GSH biosynthesis[30,31]. Lipid peroxidation (MDA) increased in the PPA-treated group, as seen in Table 1, with reported values of +20.09% higher than in the control group. This was consistent with El-Ansary *et al.*[26] findings, which indicated a significant increase in MDA in the brains of the PPA group compared to the control group.

The antioxidant efficiency of luteolin shown in the present study was confirmed by the previous work of Ueda *et al.*[32], who found that luteolin supplementation reduced lipid peroxidation and increased levels of antioxidant enzymes. Luteolin has four hydroxyl groups at the 3, 4, 5 and 7 positions, which are necessary for its antioxidant action. It has been proposed that the quantity of hydroxyl groups substituted on ring B, particularly at the 3rd position, is directly related to the free radical scavenging ability of luteolin. Catalase is an antioxidant enzyme that is present in all organisms that require oxygen. It plays a vital role in decomposing hydrogen peroxide into water and oxygen. This process protects cells from injury caused by reactive oxygen species, thereby preventing oxidative damage[33]. The data presented in Table 1 shows an elevation of catalase activity in the PPA group with recorded values of +61.00% and no significant difference compared to the control group. This result could be related to the pathological effects of PPA because similarly elevated levels of catalase were found in children with ASD[27,34]. Previous research by Alonazi *et al.*[35] found that catalase activity went down in rats treated with PPA, but the higher activity seen in rats treated with PPA does not contradict that finding. This finding is expected because it is well known that *in vivo* systems, catalase activity increases when oxidative stress is not very strong or prolonged. However, if oxidative stress is severe or persistent, it can significantly reduce catalase activity and cause severe protein damage through either direct oxidative damage to the catalase molecules, oxidative stress-induced gene expression or both[36]. This can ultimately lead to cellular dysfunction and contribute to the development of various diseases.

The current study discovered that luteolin significantly enhanced the detoxification systems of the rats that were given it. There was +140.33% higher GST activity ($p<0.0046$) in the therapeutic group (Table 1), which is a key family of enzymes that get rid of harmful compounds by connecting them to GSH, the body's main antioxidant. While PPA increased GST activity by +24.69%, the effect was not significantly different from the control. El-Ansary *et al.*[37] came to the same conclusion as this study: There was no significant difference in GST activity between the brains of rats that were given PPA and rats that were not given PPA.

According to research by Koppenol and Hider[38] and Talluri *et al.*[39], ascorbic acid, often referred to as vitamin C, is a powerful antioxidant that humans do not synthesize in their bodies and must obtain only through diet to maintain their levels of vitamin C. Table 1 shows that the luteolin treatment group had higher vitamin C levels than the control group. This was in good agreement with Zhang *et al.*[40] and Ashokkumar and Sudhandiran[41], who recorded that luteolin increases antioxidants while decreasing neurodevelopmental damage by protecting against oxidative stress. Alfawaz *et al.*[42] found that vitamin C activity was remarkably lower in rats treated with PPA which was consistent with the finding of the present study.

Under the normal physiological environment, GRP78 works as a molecular chaperone, binding to misfolded proteins and initiating ER-associated destruction of minimized misfolded proteins. On the other hand, GRP78 increases expression due to the accumulation of unfolded proteins in the ER response to ER stress[43,44]. Studies in both animal models and patients with ASD reported that ER stress is one of the underlying mechanisms of ASD pathology[13,45]. Table 1 demonstrated that GRP78 levels in PPA-treated rats are comparable to those in the control group, with no significant differences. This was consistent with Cirrik *et al.*[13] recent discovery that, whereas ER stress was linked to ASD, plasma levels of GRP78 did not change in ASD patients, which supports the current work as an experimental, preclinical study. Their findings suggested that GRP78 is not a good biomarker for detecting ER stress in ASD patients.

Table 1 shows that luteolin reduced the GRP78 level in the brain of protective-treated rats with a significant change ($p<0.0473$) and recorded values of 55.24% and in luteolin-treated rats with recorded values of 66.60% compared to the control group. The results of Kou *et al.*[46] corroborated our investigation, demonstrating that in a study using mouse models, Alzheimer's disease luteolin observed a decrease in GRP78 expression. The decrease in GRP78 expression indicates that luteolin might have an inhibitory effect on ER stress.

Caspase-3 is an essential protein involved in the process of programmed cell death, often known as apoptosis. Caspase-3 plays a crucial role in the normal development of the brain and is necessary for programmed cell death in specific cell types or response to specific death-inducing stimuli[47]. Table 1 demonstrated a significant decrease in levels of caspase-3 in the brain homogenates of PPA-treated rats ($p<0.0019$), with recorded values of 65.07% compared to controls. There was no significant difference between the therapeutic group ($p<0.8746$), with recorded values of +4.02% and the control group. A similar finding in caspase-3 has been reported by MacFabe *et al.*[48] who provided evidence that PPA treatment did not cause increased amounts of activated cell death (caspase-3). Interestingly, the observed reduction in caspase-3 in PPA-treated rats could be related to PPA-induced autistic behavioral traits reported by Abuaish *et al.*[5] and Lo *et al.*[49]. Abuaish *et al.*[5] found lower social contact in PPA-treated rats as a rodent model of autism, whereas

Cheng *et al.*[50] found poor social interaction in Caspase 3-/-males when exposed to a freely roaming novel mouse, including decreased interaction time and mounting. Thus, caspase-3 is required for a subset of social behaviors.

Table 1 showed that luteolin dramatically reduced caspase-3 levels in both the protected and therapeutic groups. This may contradicted the previous explanation linking lower caspase-3 in PPA-treated rats to impaired social interaction in a rodent model of autism[5,50], but it was consistent with the findings of Liu *et al.*[51], who discovered that luteolin reduced the protein levels of caspase-3 as a pro-apoptotic marker in cells treated with methylglyoxal. Current findings suggest that luteolin may lower oxidative stress, neuroinflammation and apoptotic cell death in mouse models of several neurological diseases[52-54]. Further studies are recommended to unveil the underlying factors responsible for the protective effects of luteolin against ASD phenotypes. Among the measured variables oxidative stress-related markers and caspase-3 recorded high AUCs, specificity and sensitivity through the use of ROC analysis showing their usefulness either as markers of PPA neurotoxicity or luteolin health-promoting effects. On the other hand, GRP78 demonstrates a useless marker of ER in PPA neurotoxicity and a good marker for luteolin protective or therapeutic potency (Table 2). Table 3 demonstrates the Pearson's correlations between the measured variables. Among the presented correlations, it is noteworthy to discuss the significant positive correlations between lipid peroxides and GRP78 as markers of oxidative and ER stress respectively. This can be supported by considering previous studies that ascertained links between oxidative stress, redox protein folding, ER stress and inflammatory responses and the development of various diseases[55]. However, despite the substantial body of evidence suggesting that PPA exposure contributes to and/or causes ASD, the present investigation's limitations include the analysis of the molecular pathways connecting the alteration of the tested markers in the PPA-treated group with the behavioral autistic features development in the rodent model.

CONCLUSION

Overall, the findings of this study suggested that PPA causes neurotoxicity-associated oxidative stress. Furthermore, studies have indicated that luteolin is neuroprotective and neurotherapeutic, making it a good candidate for treating ER, which is the genesis of neurological illnesses like autism. More research is needed to determine the efficacy of luteolin in treating various neurophenotypes in ASD, such as neuroinflammation, glutamate excitotoxicity and altered gut microbiota.

SIGNIFICANCE STATEMENT

Luteolin is likely one of the bioactive compounds that help different types of medicinal plants work as medicines to prevent disease. Since preclinical studies have shown that luteolin has pharmacological effects, including the ability to scavenge reactive oxygen species and to reverse cognitive disabilities, the current study evaluated its potential to protect and treat brain intoxication caused by PPA believed to be associated with autism and learning impairments. Luteolin use might help in treating various neurophenotypes in ASD, such as neuroinflammation, glutamate excitotoxicity and altered gut microbiota.

ACKNOWLEDGMENT

The authors express their gratitude to Research Supporting Program (RSP2024R237) of King Saud University, Riyadh, Saudi Arabia.

REFERENCES

1. Genovese, A. and M.G. Butler, 2020. Clinical assessment, genetics, and treatment approaches in autism spectrum disorder (ASD). Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21134726.
2. Volkmar, F.R. and L.A. Wiesner, 2017. Essential Clinical Guide to Understanding and Treating Autism. Wiley, Hoboken, New Jersey, United States, ISBN: 9781119427049, Pages: 368.
3. Pijl, M.K.J., J.K. Buitelaar, M.W.P. de Korte, N.N.J. Rommelse and I.J. Oosterling, 2018. Sustainability of an early detection program for autism spectrum disorder over the course of 8 years. Autism, 22: 1018-1024.
4. Nabi, M. and N. Tabassum, 2022. Role of environmental toxicants on neurodegenerative disorders. Front. Toxicol., Vol. 4. 10.3389/ftox.2022.837579.
5. Abuaish, S., N.M. Al-Otaibi, T.S. Abujamel, S.A. Alzahrani and S.M. Alotaibi *et al.*, 2021. Fecal transplant and bifidobacterium treatments modulate gut clostridium bacteria and rescue social impairment and hippocampal BDNF expression in a rodent model of autism. Brain Sci., Vol. 11. 10.3390/brainsci11081038.
6. Mohammad, F.K., M.V. Palukuri, S. Shivakumar, R. Rengaswamy and S. Sahoo, 2022. A computational framework for studying gut-brain axis in autism spectrum disorder. Front. Physiol., Vol. 13. 10.3389/fphys.2022.760753.
7. Chapman, N.H., A.Q. Nato Jr., R. Bernier, K. Ankenman and H. Sohi *et al.*, 2015. Whole exome sequencing in extended families with autism spectrum disorder implicates four candidate genes. Hum. Genet., 134: 1055-1068.

8. Abdelli, L.S., A. Samsam and S.A. Naser, 2019. Propionic acid induces gliosis and neuro-inflammation through modulation of PTEN/AKT pathway in autism spectrum disorder. Sci. Rep., Vol. 9. 10.1038/s41598-019-45348-z.
9. Meeking, M.M., D.F. MacFabe, J.R. Mepham, K.A. Foley and L.J. Tichenoff *et al*., 2020. Propionic acid induced behavioural effects of relevance to autism spectrum disorder evaluated in the hole board test with rats. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, Vol. 97. 10.1016/j.pnpbp.2019.109794.
10. El-Ansary, A., A.B. Bacha, G. Bjørklund, N. Al-Orf, R.S. Bhat, N. Moubayed and K. Abed, 2018. Probiotic treatment reduces the autistic-like excitation/inhibition imbalance in juvenile hamsters induced by orally administered propionic acid and clindamycin. Metab. Brain Dis., 33: 1155-1164.
11. MacFabe, D., 2013. Autism: Metabolism, mitochondria, and the microbiome. Global Adv. Health Med., 2: 52-66.
12. Kawada, K., S. Mimori, Y. Okuma and Y. Nomura, 2018. Involvement of endoplasmic reticulum stress and neurite outgrowth in the model mice of autism spectrum disorder. Neurochem. Int., 119: 115-119.
13. Cirrik, S., E. Esnafoglu and O. Adiguzel, 2021. Evaluation of plasma GRP78 levels in patients with autism spectrum disorder. Dusunen Adam J. Psychiatry Neurol. Sci., 34: 83-88.
14. Nabavi, S.F., N. Braidy, O. Gortzi, E.S. Sanchez, M. Daglia, K.S. Woźniak and S.M. Nabavi, 2015. Luteolin as an anti-inflammatory and neuroprotective agent: A brief review. Brain Res. Bull., 119: 1-11.
15. Xu, J., H. Wang, K. Ding, L. Zhang and C. Wang *et al*., 2014. Luteolin provides neuroprotection in models of traumatic brain injury via the Nrf2-ARE pathway. Free Radical Biol. Med., 71: 186-195.
16. Alsubaiei, S.R.M., H.A. Alfawaz, A.Y. Almubarak, N.A. Alabdali, A.B. Bacha and A. El-Ansary, 2023. Independent and combined effects of probiotics and prebiotics as supplements or food-rich diets on a propionic-acid-induced rodent model of autism spectrum disorder. Metabolites, Vol. 13. 10.3390/metabo13010050.
17. Caltagirone, C., C. Cisari, C. Schievano, R. di Paola and M. Cordaro *et al*., 2016. Co-ultramicronized palmitoylethanolamide/luteolin in the treatment of cerebral ischemia: From rodent to Man. Transl. Stroke Res., 7: 54-69.
18. Kwon, K., Y.S. Kwon, S.W. Kim, K. Yu, K.H. Lee and O.Y. Kwon, 2017. Luteolin-induced apoptosis through activation of endoplasmic reticulum stress sensors in pheochromocytoma cells. Mol. Med. Rep., 16: 380-386.
19. Maehly, A.C., 1954. The Assay of Catalases and Peroxidases. In: Methods of Biochemical Analysis, Glick, D. (Ed.), John Wiley & Sons, Hoboken, New Jersey, United States, ISBN: 9780471304265, pp: 357-424.
20. Mannervik, B., 1985. The Isoenzymes of Glutathione Transferase. In: Advances in Enzymology and Related Areas of Molecular Biology, Meister, A. (Ed.), John Wiley & Sons, Inc., Hoboken, New Jersey, United States, ISBN: 9780470123034, pp: 357-417.
21. Moron, M.S., J.W. Depierre and B. Mannervik, 1979. Levels of glutathione, glutathione reductase and glutathione *S*-transferase activities in rat lung and liver. Biochim. Biophys. Acta Gen. Subj., 582: 67-78.
22. Jagota, S.K. and H.M. Dani, 1982. A new colorimetric technique for the estimation of vitamin C using folin phenol reagent. Anal. Biochem., 127: 178-182.
23. Ruiz-Larrea, M.B., A.M. Leal, M. Liza, M. Lacort and H. de Groot, 1994. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. Steriods, 59: 383-388.
24. Bangar, S.P., P. Kajla, V. Chaudhary, N. Sharma and F. Ozogul, 2023. Luteolin: A flavone with myriads of bioactivities and food applications. Food Biosci., Vol. 52. 10.1016/j.fbio.2023.102366.
25. He, Z., X. Li, Z. Wang, Y. Cao and S. Han *et al*., 2023. Protective effects of luteolin against amyloid beta-induced oxidative stress and mitochondrial impairments through peroxisome proliferator-activated receptor γ-dependent mechanism in Alzheimer's disease. Redox Biol., Vol. 66. 10.1016/j.redox.2023.102848.
26. El-Ansary, A.K., A.B. Bacha and M. Kotb, 2012. Etiology of autistic features: The persisting neurotoxic effects of propionic acid. J. Neuroinflammation, Vol. 9. 10.1186/1742-2094-9-74.
27. Liu, X., J. Lin, H. Zhang, N.U. Khan and J. Zhang *et al*., 2022. Oxidative stress in autism spectrum disorder-current progress of mechanisms and biomarkers. Front. Psychiatry, Vol. 13. 10.3389/fpsyt.2022.813304.
28. Burke, S.L., J. Cobb, R. Agarwal, M. Maddux and M.S. Cooke, 2021. How robust is the evidence for a role of oxidative stress in autism spectrum disorders and intellectual disabilities? J. Autism Dev. Disord., 51: 1428-1445.
29. Alsubaiei, S.R.M., H.A. Alfawaz, R.S. Bhat and A. El-Ansary, 2023. Nutritional intervention as a complementary neuroprotective approach against propionic acid-induced neurotoxicity and associated biochemical autistic features in rat pups. Metabolites, Vol. 13. 10.3390/metabo13060738.
30. Kang, K.A., M.J. Piao, Y.S. Ryu, Y.J. Hyun and J.E. Park *et al*., 2017. Luteolin induces apoptotic cell death via antioxidant activity in human colon cancer cells. Int. J. Oncol., 51: 1169-1178.
31. Kang, K.A., M.J. Piao, Y.J. Hyun, A.X. Zhen and S.J. Cho *et al*., 2019. Luteolin promotes apoptotic cell death via upregulation of Nrf2 expression by DNA demethylase and the interaction of Nrf2 with p53 in human colon cancer cells. Exp. Mol. Med., 51: 1-14.
32. Ueda, H., C. Yamazaki and M. Yamazaki, 2002. Luteolin as an anti-inflammatory and anti-allergic constituent of *Perilla frutescens*. Biol. Pharm. Bull., 25: 1197-1202.
33. Kehrer, J.P., J.D. Robertson and C.V. Smith, 2010. Free Radicals and Reactive Oxygen Species. In: Comprehensive Toxicology, McQueen, C.A. (Ed.), Elsevier, Amsterdam, Netherlands, ISBN: 9780080468846, pp: 277-307.

34. Usui, N., H. Kobayashi and S. Shimada, 2023. Neuroinflammation and oxidative stress in the pathogenesis of autism spectrum disorder. Int. J. Mol. Sci., Vol. 24. 10.3390/ijms24065487.
35. Alonazi, M., A.B. Bacha, M.G. Alharbi, A.I.A. Khayyat, L. AL-Ayadhi and A. El-Ansary, 2023. Bee pollen and probiotics' potential to protect and treat intestinal permeability in propionic acid-induced rodent model of autism. Metabolites, Vol. 13. 10.3390/metabo13040548.
36. El-Ansary, A.K., S. Al-Daihan, A.B. Bacha, G.H. Shaker and L.Y. Al-Ayadhi, 2013. Comparative study on the protective effect of carnosine and carnitine against pro-inflammatory/pro-oxidant effects of clindamycin and propionic acid administrations to hamsters. Afr. J. Microbiol. Res., 7: 103-114.
37. El-Ansary, A., R.S. Bhat, S. Al-Daihan and A.M. Al Dbass, 2015. The neurotoxic effects of ampicillin-associated gut bacterial imbalances compared to those of orally administered propionic acid in the etiology of persistent autistic features in rat pups: Effects of various dietary regimens. Gut Pathog., Vol. 7. 10.1186/s13099-015-0054-4.
38. Koppenol, W.H. and R.H. Hider, 2019. Iron and redox cycling. Do's and don'ts. Free Radical Biol. Med., 133: 3-10.
39. Talluri, R.S., S. Katragadda, D. Pal and A.K. Mitra, 2006. Mechanism of L-ascorbic acid uptake by rabbit corneal epithelial cells: Evidence for the involvement of sodium-dependent vitamin C transporter 2. Curr. Eye Res., 31: 481-489.
40. Zhang, M., W. Liu, Y. Zhou, Y. Li, Y. Qin and Y. Xu, 2018. Neurodevelopmental toxicity induced by maternal PM2.5 exposure and protective effects of quercetin and vitamin C. Chemosphere, 213: 182-196.
41. Ashokkumar, P. and G. Sudhandiran, 2008. Protective role of luteolin on the status of lipid peroxidation and antioxidant defense against azoxymethane-induced experimental colon carcinogenesis. Biomed. Pharmacother., 62: 590-597.
42. Alfawaz, H.A., A. El-Ansary, L. Al-Ayadhi, R.S. Bhat and W.M. Hassan, 2022. Protective effects of bee pollen on multiple propionic acid-induced biochemical autistic features in a rat model. Metabolites, Vol. 12. 10.3390/metabo12070571.
43. Kaushik, S. and A.M. Cuervo, 2015. Proteostasis and aging. Nat. Med., 21: 1406-1415.
44. Lee, A.H., N.N. Iwakoshi and L.H. Glimcher, 2003. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol. Cell. Biol., 23: 7448-7459.
45. Ibrahim, I.M., D.H. Abdelmalek and A.A. Elfiky, 2019. GRP78: A cell's response to stress. Life Sci., 226: 156-163.
46. Kou, J.J., J.Z. Shi, Y.Y. He, J.J. Hao and H.Y. Zhang *et al*., 2022. Luteolin alleviates cognitive impairment in Alzheimer's disease mouse model via inhibiting endoplasmic reticulum stress-dependent neuroinflammation. Acta Pharmacol. Sin., 43: 840-849.
47. Porter, A.G. and R.U. Janicke, 1999. Emerging roles of caspase-3 in apoptosis. Cell Death Differ., 6: 99-104.
48. MacFabe, D.F., K. Rodriguez-Capote, J.E. Hoffman, A.E. Franklin and Y. Mohammad-Asef *et al*., 2008. A novel rodent model of autism: Intraventricular infusions of propionic acid increase locomotor activity and induce neuroinflammation and oxidative stress in discrete regions of adult rat brain. Am. J. Biochem. Biotehcnol., 4: 146-166.
49. Lo, S.C., K. Scearce-Levie and M. Sheng, 2016. Characterization of social behaviors in caspase-3 deficient mice. Sci. Rep., Vol. 6. 10.1038/srep18335.
50. Cheng, H.Y., M.T. Hsieh, F.S. Tsai, C.R. Wu and C.S. Chiu *et al*., 2010. Neuroprotective effect of luteolin on amyloid β protein (25-35)-induced toxicity in cultured rat cortical neurons. Phytother. Res., 24: S102-S108.
51. Liu, Y., J. Huang, X. Zheng, X. Yang and Y. Ding *et al*., 2017. Luteolin, a natural flavonoid, inhibits methylglyoxal induced apoptosis via the mTOR/4E-BP1 signaling pathway. Sci. Rep., Vol. 7. 10.1038/s41598-017-08204-6.
52. Lin, T.Y., C.W. Lu and S.J. Wang, 2016. Luteolin protects the hippocampus against neuron impairments induced by kainic acid in rats. NeuroToxicology, 55: 48-57.
53. Ahmad, S., M.H. Jo, M. Ikram, A. Khan and M.O. Kim, 2021. Deciphering the potential neuroprotective effects of luteolin against $A\beta_{1-42}$-induced Alzheimer's disease. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22179583.
54. Nishiguchi, H., T. Omura, A. Sato, Y. Kitahiro, K. Yamamoto, J. Kunimasa and I. Yano, 2024. Luteolin protects against 6-hydoroxydopamine-induced cell death *via* an upregulation of HRD1 and SEL1L. Neurochem. Res., 49: 117-128.
55. Victor, P., D. Sarada and K.M. Ramkumar, 2021. Crosstalk between endoplasmic reticulum stress and oxidative stress: Focus on protein disulfide isomerase and endoplasmic reticulum oxidase 1. Eur. J. Pharmacol., Vol. 892. 10.1016/j.ejphar.2020.173749.