VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 602-612 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Effect of Tetrahydrocurcumin Improves Erythromycin Estolate Induced Alterations of Acetylcholinesterase, ATPases and Oxidative Stress in Brain of Rats

Murugan Pidaran

Department of Biochemistry, Centre for Distance and Online Education, Bharathidhasan University, Tiruchirapalli – 620024, Tamil Nadu, India

Received: 16th February, 2024; **Accepted:** 6th June, 2024; **Published online:** 4th September, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.057

Abstract: Curcumin is a phenolic compound extracted from *Curcuma longa* rhizome commonly used in Asia as a spice, pigment and additive. In traditional medicine of India and China, curcumin is considered as a therapeutic agent used in several foods. Numerous studies have shown that curcumin has broad biological functions particularly antioxidant and anti-inflammatory. Tetrahydrocurcumin (THC), produced from curcumin by hydrogenation, is colourless which render these products useful in non-colored food and cosmetic applications that currently employ synthetic antioxidants. THC is one of the major metabolites of curcumin, with potential bioactivity and is a potent antioxidant than the commonly used synthetic antioxidants, butylated hydroxytoluene. It generally has stronger inhibitory effect than curcumin *in vivo* system. Curcuminoids and THC were shown to be effective antioxidant. Their efficacy is concentration dependent, THC being effective even at lower concentration. Erythromycin estolate (EME), a potent macrolide antibiotic, generates free radicals, but their role in the development of liver toxicity is not yet well understood. The present study was carried out to investigate the effect of THC and curcumin against erythromycin estolate (EME) (800 mg/kg b wt)-induced changes in the activity of acetylcholinesterase (AChE), membrane bound enzymes, lipid peroxidation (LPO) and antioxidant status in the brain of rats. Oral administration of THC (80 mg/kg b wt) and curcumin (80 mg/kg b wt) significantly prevented the occurrence of EME induced brain damage. Oral administration of EME (800 mg/kg/day) with THC and curcumin significantly ($P < 0.05$) diminished the levels of LPO and protein carbonyls and significantly ($P<0.05$) increased the activities of ATPases, antioxidant enzymes, GSH and TSH in brain. These results indicate that THC and curcumin attenuate the LPO and alteration of antioxidant and membrane bound enzymes in EMS exposed rats, which suggest that THC and curcumin protects the brain function from toxic effects of EMS. The effect of THC was more prominent compared with curcumin.

Keywords: Tetrahydrocurcumin, Curcumin, Erythromycin estolate, ATPases, Antioxidants, Lipid peroxidation, Acetylcholinesterase

Citation: Murugan Pidaran: Effect of tetrahydrocurcumin improves erythromycin estolate induced alterations of acetylcholinesterase, ATPases and oxidative stress in brain of rats. Intern. J. Zool. Invest. 10(2): 602-612, 2024.

https://doi.org/10.33745/ijzi.2024.v10i02.057

Introduction

Neurotoxicity is a form of toxicity in which a biological, chemical, or physical agent produces an adverse effect on the structure or function of the central and/or peripheral nervous system. It occurs when exposure to a substance– specifically, a neurotoxin or neurotoxicant– alters the normal activity of the nervous system in such a way as to cause permanent or reversible damage to nervous tissue (Cunha-Oliveira *et al*., 2008). This can eventually disrupt or even kill neurons, which are cells that transmit and process signals in the brain and other parts of the nervous system. Neurotoxicity can result from organ transplants, radiation treatment, certain drug therapies, recreational drug use, exposure to heavy metals, bites from certain species of venomous snakes, pesticides (Keifer *et al*., 2007; Costa *et al*., 2008), certain industrial cleaning solvents (Sainio *et al*., 2015), fuels (Ritchie *et al*., 2001) and certain naturally occurring substances. Symptoms may appear immediately after exposure or be delayed. They may include limb weakness or numbness, loss of memory, vision, and/or intellect, uncontrollable obsessive and/or compulsive behaviors, delusions, headache, cognitive and behavioral problems and sexual dysfunction. Chronic mold exposure in homes can lead to neurotoxicity which may not appear for months to years of exposure (Curtis *et al*., 2004). All symptoms listed above are consistent with mold mycotoxin accumulation (Kilburn *et al*., 2004).

Antibiotics are among the most frequently used pharmaceuticals in both the inpatient and outpatient setting. While these antimicrobial agents are generally well tolerated, these drugs are not without their associated side effects, both dose-dependent and idiosyncratic in nature. While diarrhoea is a commonly associated adverse effect of many antibiotics, toxic effects on the central nervous system are perhaps much less recognized (Snavely and Hodges, 1984). A danger for clinicians and patients alike, of not recognizing neurotoxic effects of antibiotics is that the neurological manifestations of toxicity may be confused with a different neurological condition. Correspondingly, in cases of drug-induced encephalopathy, change in mental status may be ascribed to a metabolic abnormality especially in hospitalized patients. With greater education regarding these neurotoxic effects, medical care providers can learn to recognize toxic effects more readily and make medication adjustments as necessary since it is often a readily reversible process. A high degree of suspicion is also essential for clinicians.

Erythromycin estolate (EME) was for many years considered to be the only erythro-mycin derivative which produced jaundice; however, it is now clear that other erythromycin derivatives can also cause the same injury and can produce the same syndrome and lesion. Liver injury develops about 1 to 3 weeks after starting the medication, with high values for ALP and modestly elevated transaminase values. Jaundice and right upper quadrant pain may develop. Liver biopsy usually shows zone 3 cholestasis and prominent portal inflammatory infiltration, often rich in eosinophils (Pari and Murugan, 2004; Murugan and Pari, 2005).

Currently available drug regimes for management of diabetes mellitus have certain drawbacks and, therefore, there is a need for safer and more effective hepatoprotective drugs (Murugan, 2015 a-d). Natural products from medicinal plants continue to form a common platform for the discovery of new chemical entities in the modern drug discovery programmes (Murugan, 2021 a, b). The belief that natural medicines are much safer than synthetic drugs has gained popularity in recent years and lead to tremendous growth of phytopharma-ceutical usage (Murugan, 2023 a). A wide array of plant derived active principles (phytochemicals) for possible use in the treatment of hepatoprotective effect has been reported (Bailey and Day, 1989).

Curcuma longa is commonly used in the treatment of diabetes by ayurvedic physicians. Curcumin is a biologically active component

isolated from the rhizome of *Curcuma longa* that possess antihyperglycemic activity (Murugan, 2010; Murugan, 2023b), hypolipidemic action (Murugan, 2021c) and anti-renal lesion effect (Murugan and Sakthivel, 2021; Chaudhary *et al.*, 2024). The use of curcumin is recommended for prevention of advanced glycated endproducts (AGE) accumulation and the associated complications of diabetes (Murugan and Maneemegalai, 2024).

THC is one of the major colourless metabolite of curcumin. THC has been reported to exhibit the same physiological and pharmacological properties of curcumin (Murugan, 2022). Curcumin is rapidly metabolized during absorption from the intestine, yielding THC (Murugan, 2023c), which has shown the strongest antioxidant activity among all curcuminoids (Murugan, 2023d). Several studies in experimental animals indicated that THC also prevent(s) cancer (Lin and Lin-Shiau, 2001), protect(s) against inflammation (Hong *et al*., 2004), atherosclerotic lesions (Naito *et al*., 2002), hepatotoxicity (Pari and Murugan, 2004) and nephrotoxicity (Murugan and Pari, 2005). There is no other report available in the literature on the protective effect of THC compared curcumin against EME-induced brain damage on oxidative stress. The present study was carried out to investigate the effect of THC and curcumin against erythromycin estolate (EME) (800 mg/kg b wt)-induced changes in the activity of acetylcholinesterase (AChE), membrane bound enzymes, lipid peroxidation (LPO) and antioxidant status in the brain of rats.

Materials and Methods

Chemicals:

THC (95.27% purity) was a gift provided by Sabinsa Corporation, USA. EME was purchased from Ipca Laboratories Ltd., Ratlam, India and curcumin from the Daiwa Kasei Co., Ltd., Saitama, Japan. Thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), total glutathione, 2,2-dipyridyl, xylenol orange, aspartic acid, alanine, a-ketoglutarate (a-KG), 2,4- dinitro phenyl hydrazine (DNPH) and 5,5o-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were obtained from a local firm (India) and were of highest purity grade.

Animals:

Adult male albino Wistar rats (8 weeks), weighing 180 to 200 g bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used. All animal experiments were approved by the Ethical Committee, Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The animals were housed in polycarbonate cages in a room with a 12 h day-night cycle, temperature of 24 ± 2° C, and humidity of 45 to 64%. During the whole experimental period, animals were fed with a balanced commercial diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Experimental design:

Animals were randomized and divided into 4 groups (n=6 in each group). Animals in Group I (untreated control), Group II animals received a single oral administration of EME (800 mg/kg b wt) (Pari and Murugan, 2006). Animals from group III received EME and THC (80 mg/kg b wt) for 8 days orally before the single oral administration of EME, and treatment with THC followed for 7 more days by intragastric entubation. Group IV received EME and curcumin (80 mg/kg b wt) for 8 days orally before the single oral administration of EME and treatment with curcumin followed for 7 more days by intragastric entubation. After the experimental period, the animals in the different groups were sacrificed. Blood was collected in two different tubes i.e. one with anticoagulant for plasma separation and another without anticoagulant to separate serum for various biochemical estimations. The Brain were extirpated, washed in ice-cold saline, blotted dry and weighed. Then 10% brain homogenate was prepared in phosphate buffer 0.1 M, pH 7.4

and used for the biochemical analysis.

Analytical methods:

Determination of acetylcholinesterase activity:

Acetylcholinesterase (AChE) activity was determined in plasma and brain using acetylthiocholine iodide as a substrate according to the method of Ellman *et al*. (1961). In this method, AChE in samples hydrolyzes acetylthiocholine iodide into thiocholine and butyric acid. The thiocholine reacts with 5,5-dithiobis (2-nitrobenzoic acid) to form 5-thio-2-nitrobenzoic acid. The yellow colour developed was measured spectrophotometrically at 412 nm.

Estimation of LPO and protein carbonyl contents:

LPO in brain was determined by measuring the levels of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) by the method of Niehaus and Samuelsson (1968) and Jiang *et al*. (1992), respectively. The level of protein carbonyl in brain homogenate was determined by the method of Levine *et al*. (1990).

Determination of antioxidant activities:

The levels of reduced glutathione (GSH) in brain homogenate was determined by the method of Moron *et al*. (1979) based on the reaction with Ellman's reagent (19.8 mg DTNB in 100 ml of 0.1% sodium citrate). The levels of total sulphydryl groups (TSH) was measured after the reaction with 5,5-dithiobis (2-nitrobenzoic acid) using the method of Ellman (1959). The activities of superoxide dismutase (SOD) (Kakkar *et al*., 1984), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPx) (Rotruck *et al*., 1973) and glutathione-*S*-transferase (GST) (Habig *et al*., 1974) were also measured spectrophotometrically in brain homogenate. The protein concentration in brain homogenate was determined by the method of Lowry *et al*. (1951) using bovine serum albumin as a standard.

Determination of ATPase activities:

Total ATPase activity in brain homogenate was measured by the method of Evans (1969). The ATPase activity in 0.1 ml aliquot of the homogenates were measured in a final volume of 2ml containing 0.1 ml of 0.1 M Tris–HCl (pH 7.4), 0.1 ml of 0.1 M NaCl, 0.1 ml of 0.1 M $MgCl_2$, 1.5 ml of 0.1 M KCl, 0.1 ml of 1 mM EDTA and 0.1 ml of 0.01 M ATP. The reaction was stopped at 20 min by the addition of 1 ml of 10% TCA and then centrifuged (1500×*g* for 10 min), and the inorganic phosphorus (Pi) liberated was estimated in the protein-free supernatant. The amount of liberated Pi was estimated according to the method of Fiske and Subbarow (1925) using commercial diagnostic kit (Qualigens diagnostics, India).

The activity of Na+/K+-dependent ATPase was determined by the method of Bonting (1970). In this assay, 0.2 ml of brain tissue homogenate was added to the mixture containing 1 ml of 184 mM Tris–HCl buffer (pH 7.5), 0.2 ml of 50 mM $MgSO_4$, 0.2 ml of 50 mM KCl, 0.2 ml of 600 mM NaCl, 0.2 ml of 1 mM EDTA and 0.2 ml of 10 mM ATP and incubated for 15 min at 37 °C. The reaction was arrested by the addition of 1 ml of ice cold 10% TCA. Then the amount of Pi liberated was estimated in protein free supernatant. The activity of Ca^{2+}- ATPase was assayed according to the method of Hjertan and Pan (1983). In brief, 0.1 ml of tissue homogenate was added to a mixture containing 0.1 ml of 125 mM Tris–HCl buffer (pH 8), 0.1 ml of 50 mM $CaCl_2$ and 0.1 ml of 10 mM ATP. The contents were incubated at 37 °C for 15 min. The reaction was then arrested by the addition of 0.5 ml of ice cold 10% TCA and centrifuged. The amount of Pi liberated was estimated in supernatant. The activity of Mg^{2+}-ATPase was assayed by the method of Ohinishi *et al*. (1982). The incubation mixture contained 0.1 ml of 375 mM Tris–HCl buffer (pH 7.6), 0.1 ml of 25 mM $MgCl_2$, 0.1 ml of 10 mM ATP, 0.1 ml water and 0.1 ml of tissue homogenate. The contents were incubated for 15 min at 37°C and the reaction was arrested by adding 0.5 ml of 10% TCA. The Pi liberated was then estimated in protein free supernatant. The activities of these ATPase enzymes in tissue homogenate were expressed as mg Pi liberated/min/mg protein.

Table 1: Changes in the activities of acetylcholinesterase (AChE) in plasma and brain of control and experimental rats

Groups	Plasma (µmol of ATCI Hydrolysed/min/mg protein)	Brain (µmol of ATCI Hydrolysed/min/mg protein)
Control	2.61± 0.12[a]	16.35 ± 0.78 [a]
EME (800 mg/kg)	1.58± 0.10[b]	8.48 ± 0.48 [b]
EME (800 mg/kg) +THC (80 mg/kg)	2.46± 0.13[c]	14.21±0.58[c]
EME (800 mg/kg) + curcumin (80 mg/kg)	2.35± 0.13[d]	13.01±0.45[d]

ATCI: acetyl thiocholine iodide; CON: control; Values are mean ± S.D. for six rats in each group. Values not sharing a common letter differ significantly at P < 0.05 (DMRT).

Table 2: Effect of THC and curcumin and EME on the levels of lipid peroxidation, protein carbonyl contents, reduced gluathione (GSH) and total sulphydryl groups in brain of control and experimental rats

Parameters	Control	EME (800 mg/kg)	EME(800 mg/kg)+THC (80 mg/kg)	EME(800 mg/kg)+curcumin (80 mg/kg)
TBARS(µmol/g tissue)	12.68±0.66[a]	19.41±1.20[b]	14.19±0.85[c]	16.78±0.75[d]
LOOH(mmol/g tissue)	1.32±0.05[a]	1.89±0.13[b]	1.24±0.87[c]	1.39±0.78[d]
Protein carbonyls(nmol/mg protein)	4.05±0.35[a]	8.32±0.78[b]	4.98±0.51[c]	5.89±0.51[d]
GSH(µg/mg protein)	3.15±0.16[a]	1.55±0.13[b]	2.72±0.32[c]	2.35±0.23[d]
TSH(µg/mg protein)	8.25±0.51[a]	5.31±0.45[b]	7.19±0.41[c]	6.72±0.50[d]

TBARS: thiobarbituric acid reactive substances; LOOH: lipid hydroperoxides. Values are mean ± S.D. for six rats in each group. Within rows, means with different superscript letters (a, b and c) differ significantly at P<0.05 (DMRT).

Results

Previous studies in our laboratory showed that THC has protective effect on EME induced toxicity at a dose of 80 mg/kg b wt (Pari and Murugan, 2004). To determine whether the THC and Curcumin has similar effect in EME induced oxidative injury in brain, this study was performed with a fixed dose of THC and curcumin (80 mg/kg b wt). The activity of AChE in plasma and brain of control and experimental rats are shown in Table 1. The activities of AChE in brain and plasma was significantly (P<0.05) decreased in EME intoxicated rats whereas the administration of THC and curcumin (80 mg/kg b wt) significantly (P < 0.05) reversed the activities of AChE to near normal in EME exposed rats.

Effect on brain lipid peroxidation and antioxidants:

Table 2 shows the changes in the levels of lipid peroxidation and the activities of antioxidant enzymes in normal and experimental rats. TBARS and hydroperoxides from brain homogenate were significantly decreased with THC treatment whereas; EME treated rats showed significantly increased levels of lipid peroxidation products. The effect of THC was better than curcumin.

Table 3 demonstrates the changes in the activities of antioxidant enzymes and ATPase enzymes (Na+K+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase) in brain of control and experimental rats. A significant (P < 0.05) decrease in the activities of SOD, CAT, GPx, GST and ATPases enzymes were observed in EME alone treated rats in comparison with control rats. Treatment with THC and curcumin significantly (P < 0.05) increased the

Table 3: Effect of THC and curcumin and EME on antioxidant enzymes and ATPases in brain of control and experimental rats

Parameters	Control	EME (800 mg/kg)	EME (800 mg/kg)+THC (80 mg/kg)	EME(800 mg/kg) + curcumin (80 mg/kg)
Antioxidant enzymes SOD	9.25±0.72[a]	6.79±0.52[b]	8.36±0.65[c]	7.12±0.54[d]
CAT	4.74±0.36[a]	2.61±0.20[b]	3.89±0.17[c]	3.35±0.18[d]
GPx	3.79±0.21[a]	2.45±0.17[b]	3.45±0.14[c]	3.17±0.13[d]
GST	6.32±0.28[a]	4.29±0.20[b]	5.81±0.26[c]	5.35±0.20[d]
Membrane bound ATPases	1.53±0.18[a]	1.08±0.10[b]	1.38±0.13[c]	1.16±0.12[d]
Total ATPases				
Na^+K^+-ATPase	**0.38±0.03[a]**	**0.22±0.02[b]**	**0.33±0.02[c]**	**0.30±0.03[d]**
Ca^{2+}-ATPase	0.43±0.05[a]	0.28±0.02[b]	0.39±0.04[c]	0.36±0.03[d]
Mg^{2+}-ATPase	0.30±0.03[a]	0.21±0.02[b]	0.28±0.02[c]	0.26±0.03[d]

Values are mean ± S.D. for six rats in each group. Within rows, means with different superscript letters (a, b and c) differ significantly at $P<0.05$ (DMRT). The activities of enzymes are expressed as follows: SOD, one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of nitroblue tetrazolium reduction in 1 min/mg protein; CAT, micromoles of hydrogen peroxide consumed/min; GPx, microgram of glutathione consumed/min/mg protein; GST, micromoles of 1-chloro 2,4-dinitrobenzene (CDNB)-GSH conjugate formed/min/mg protein. The activities of ATPase enzymes were expressed as μg Pi liberated/min/mg protein.

activities of antioxidant and ATPases enzymes in brain when compared to EME alone treated rats.

Discussion

Numerous studies demonstrate that changes in the antioxidant system and impairment of sodium and potassium transport are essential factors involved in the brain tissue disturbance in various kinds of oxidative injury in the central nervous system (Jenner, 1998; Slyshenkov *et al*., 2002). Epilepsy is a chronic neurological disorder that affects more than 70 million people worldwide, with a generally bimodal incidence that mainly affects children and the elderly (Thijs *et al*., 2019). It is characterized by a persistent propensity to develop epileptic seizures due to abnormal excessive or synchronous neuronal activity in the brain (Fisher *et al*., 2005). The molecular mechanisms underlying the development of epilepsy are related to neural injury resulting from several factors, including oxidative stress (OS) in neural tissues (Mao *et al*., 2019).

AChE is responsible for degrading the acetylcholine neurotransmitter into choline and acetic acid, bringing an end point to cholinergic neurotransmission (Murugan, 2021 d, e). This enzyme is heterogeneously distributed in the brain showing different activity depending on the nervous region. Too much acetylcholine can lead to what is known as a cholinergic crisis. This can happen from external causes such as from taking high-dose AChE inhibitors, or from exposure to something like pesticides, or insecticides. Symptoms include: blurred vision (Alkalay *et al*., 2013).

Oxidative stress occurs when the antioxidant defense system, consisting of endogenous antioxidants, such as catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), and reduced glutathione (GSH), and a variety of exogenous antioxidants obtained from the diet, cannot eliminate excess reactive species (RS) which are the result of cellular metabolism and/or external factors (Checa and Aran, 2020;Yang, *et al*., 2021).

The combination of excessive reactive oxygen species (ROS) production with nitric oxide (NO) to form peroxynitrite, a reactive nitrogen species,

leads to lipid peroxidation and direct protein damage, which alters membrane and cell functions (Szabó *et al*., 2007; Kovac *et al*., 2017). This fact makes the brain highly susceptible to oxidative stress because of its high metabolism and high concentrations of unsaturated fatty acids (Cobley *et al*., 2018).

As a consequence of oxidative metabolism and lipid peroxidation, damage occurs to the constituents of neuronal membranes, including Na,K-ATPase (NKA) (McCoy *et al*., 2019). This enzyme maintains the electrical activity of excitable cells and is essential for the maintenance of cellular homeostasis, as it is responsible for the ionic composition within the cells and osmotic balance (Farrell *et al*., 2017). Consequently, failures in the NKA activity can interfere with cell excitability and facilitate the onset or propagation of a seizure (De Freitas, 2010).

In order to improve antioxidant defense, antioxidant therapy can be performed through the ingestion of substances with antioxidant activity, either through an adequate diet or through supplementation (Barbosa *et al*., 2010). Several studies report benefits with the use of antioxidant therapy in animal models of neurological disorders (Barbosa *et al*., 2010; Kasote *et al*., 2013; Lv *et al*., 2017) and in human studies (Shinto *et al*., 2014; Taghizadeh *et al*., 2017).

Increasing evidences suggest that the excessive production of free radicals in brain, and the imbalance between oxidative species and antioxidant defenses is related to the pathogenesis of neurodegenerative diseases (Schulz *et al*., 2000). EME can penetrate the blood brain barrier and accumulate into the brain, which is easily susceptible to EME-induced LPO (Murugan and Pari, 2005). In the present study, the elevation of LPO and increased formation of protein carbonyls with reduced levels of TSH in EME intoxicated rat brain might be due to over production of free radicals and LPO end products, which leads to oxidative modifications of proteins. Numerous studies showed that the activity of antioxidant defense systems are inhibited or diminished in rats exposed to EME (Dominique Pateron *et al*., 2011; Hee Kyong *et al*., 2017). GSH is the most abundant non-protein thiol that maintains the cellular redox status and providing first line of antioxidant protection against oxidative stress in the brain tissue (Dringen *et al*., 2000). The depletion of GSH and TSH levels upon EME intoxication could be due to increased utilization to overwhelm the free radicals and LPO products in brain. The decrease in the activities of antioxidant enzymes might be due to binding of EME with sulphydryl groups of enzymes and oxidative modifications of amino acid chains, which alters the enzyme structure and leads to inactivation or decreased activity of enzyme. Thus, the decreased activities of brain antioxidant systems indicates the accumulation of free radicals and increased level of LPO, which increase the oxidative threat in tissue.

Vitamin C (ascorbate, ascorbic acid) is a potent antioxidant. Ascorbate directly scavenges ROS and nitrogen-based radicals. It also upregulates eNOS and downregulates NOX. Its antioxidant properties are intensified in combination with other antioxidants, such as vitamin E. Vitamin C plasma concentrations high enough to be able to effectively scavenge ROS can only be achieved by intravenous supplementation (Rodrigo *et al*., 2013). Higher vitamin C serum concentration was associated with a reduced risk of incidence of ischemic and hemorrhagic stroke (Morelli *et al*., 2020). The oral supplementation was shown to slightly reduce lipid peroxidation markers (Rodrigo *et al*., 2013).

An association between vitamin C and vitamin E plasma concentrations has been shown. Vitamin C has the ability to “recycle” α-tocopherol in lipid bilayers and erythrocytes, thus increasing the vitamin E antioxidant capacity (Cheng *et al*., 2018). The brain is a lipid-rich environment, so recycling vitamin E may be an important function of ascorbic acid. When stroke patients were given both vitamin C and vitamin E supplements, there was a reduction of lipid peroxidation and exerted anti-inflammatory effects (Cheng *et al*., 2018).

Na+ K+-ATPase is a key enzyme implicated in neural excitability, metabolic energy production, as well as in the uptake and release of catecholamines (Bogdanski *et al*., 1968; Mata *et al*., 1980) and serotonin (Hernandez, 1989). Moreover, the role of Mg^{2+}-ATPase is to maintain the high intracellular Mg^{2+} level in brain, changes of which can control the rate of protein synthesis and cell growth (Sanui and Rubin, 1982). Ca^{2+} functions as second a second messenger in the central nervous system. The alterations in Ca^{2+} level also leads to several pathological lesions in brain. The Ca^{2+} overload mediated by EME also inhibit the Ca^{2+} ATPase activity in cell membrane and eventually potentiates the irreversible cell destruction (Joseph *et al*., 2001). The activities of these ATPase enzymes are affected by the exposure of EME (El-Missiry and Shalaby, 2000; Antonio *et al*., 2003) indicating the alterations in membrane and neurotransmitter functions. The decreased activity of ATPases could be result from the formation of EME-ATPase complexes through SH group of enzyme and/or increased oxidative stress.

Co-administration of THC and curcumin with EME exposure restored the activities of ATPases, which reveals that THC and curcumin preserve the integrity of cellular membrane and normal physiological functions of brain. This study demonstrated that THC and curcumin protect the free radical generation, mitochondrial function and apoptotic effect of EME (Pari and Murugan, 2004; Murugan and Pari, 2005). The inhibition of radical generation with preservation of mitochondrial function and reduction of EME accumulation lead by THC and curcumin might contribute to the enhancement of ATPases activity in EME intoxicated rats treated with THC and curcumin.

Conclusion

The present investigation indicates that THC and curcumin exerts significant protection against EME induced toxicity by its ability to ameliorate the lipid peroxidation through the free radicals scavenging activity, which enhanced the levels of antioxidant defense system. This study also showed that THC has greater effect than curcumin, in EME induced lipid peroxidation. Therefore, THC should be considered as a therapeutic agent for the brain injury associated with metal induced oxidative stress.

References

Alkalay A, Rabinovici GD, Zimmerman G, Agarwal N, Kaufer D, Miller BL, Jagust WJ, Soreqd H. (2013) Plasma acetylcholinesterase activity correlates with intracerebral β-amyloid load. Curr Alzheimer Res. 10(1): 48-56.

Antonio MT, Corredor L and Leret ML. (2003) Study of the activity of several brain enzymes like markers of the neurotoxicity induced by perinatal exposure to lead and/or cadmium. Toxicol Lett. 143: 331-340.

Barbosa K, Costa N, Alfenas R, Paula DE, Minim S, Bressan V and Revista de Nutrição J. (2010) Oxidative stress: Concept, implications and modulating factors. Rev Nutr. 23: 629-643.

Bogdanski DF, Tissuri A and Brodie BB. (1968) Role of sodium, potassium, ouabain and reserpine in uptake, storage and metabolism of biogenic amines in synaptosomes. Life Sci. 7: 419-428.

Bonting SL. (1970) Presence of enzyme system in mammalian tissues. In: Membrane and Ion Transport, (ed.) Bilter E.E., Wiley Inter. Science, London, pp. 257-263.

Chaudhary K, Chand D, Kushwaha VB and Srivastav SK. (2024) Efficacy of turmeric (*Curcuma longa*) extract and selenium against *Nerium indicum* induced toxicity in the kidney of Wistar rats**.** Intern J Zool Invest. 10(2): 572-581.

Checa J and Aran JM. (2020) Reactive oxygen species: Drivers of physiological and pathological processes. J Inflamm Res. 2020: 1057-1073.

Cheng P, Wang L, Ning S, Liu Z, Lin H, Chen S and Zhu J. (2018) Vitamin E intake and risk of stroke: A meta-analysis. Br J Nutr. 120: 1181-1188.

Cobley JN, Fiorello ML and Bailey DM. (2018) 13 Reasons why the brain is susceptible to oxidative stress. Redox Biol. 15: 490-503.

Costa Lucio G, Giordano G, Guizzetti M and Vitalone A. (2008) Neurotoxicity of pesticides: a brief review. Front Biosci. 13(13): 1240-1249.

Cunha-Oliveira T, Rego AC and Oliveira CR. (2008) Cellular and molecular mechanisms involved in the neurotoxicity of opioid and psychostimulant drugs. Brain Res Rev. 58(1):192-208.

Curtis L, Lieberman A, Stark M, Rea W and Vetter M.

(2004) Adverse health effects of indoor molds. J Nutritional Environ Med. 14 (3): 261-274.

De Freitas RM. (2010) Lipoic acid alters δ-aminolevulinic dehydratase, glutathione peroxidase and Na+,K+-ATPase activities and glutathione-reduced levels in rat hippocampus after pilocarpine-induced seizures. Cell Mol Neurobiol. 30: 381-387.

Dringen R, Gutterer JM and Hirrlinger J. (2000) Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. Eur J Biochem. 267: 4912-4916.

Ellman GC. (1959) Tissue sulfhydryl groups. Arch Biochem Biophys. 82: 70-77.

El-Missiry MA and Shalaby F. (2000) Role of beta-carotene in ameliorating the cadmium-induced oxidative stress in rat brain and testis. J Biochem Mol Toxicol. 14: 238-243.

Evans Jr DJ. (1969) Membrane adenosine triphosphatase of *E. coli* activation by calcium ions and inhibition by monovalent cations. J Bacteriol. 100: 914-922.

Farrell JS, Wolff MD and Teskey GC. (2017) Neurodegeneration and pathology in epilepsy: Clinical and basic perspectives. Adv Neurobiol. 15: 317-334.

Fisher RS, Van Emde Boas W, Blume W, Elger C, Genton P, Lee P and Engel J. (2005) Epileptic seizures and epilepsy: Definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 46: 470-472.

Fiske CH and Subbarow Y. (1925) The colorimetric determination of phosphorus. J Biol Chem. 66: 375-400.

Habig WH, Pabst MJ and Jakpoby WB. (1974) Glutathione transferase, a first enzymatic step in mercapturic acid formation. J Biol Chem. 249: 7130-7139.

Haghnejad Azar A, Oryan S, Bohlooli S and Panahpour H. Alpha-tocopherol reduces brain edema and protects blood-brain barrier integrity following focal cerebral ischemia in rats. Med Princ Pract. 26: 17-22.

Hee Kyong Na, Hwoon-Yong Jung, Dong Woo Seo, Hyun Lim, Ji Yong Ahn, Jeong Hoon Lee, Do Hoon Kim, Kee Don Choi, Ho June Song, Gin Hyug Lee and Jin-Ho K. (2017) Erythromycin infusion prior to endoscopy for acute nonvariceal upper gastrointestinal bleeding: a pilot randomized controlled trial. Korean J Intern Med. 32(6):1002-1009.

Hernandez R. (1989) Brain Na^+,K^+-ATPase activity possibly regulated by a specific serotonin receptor. Brain Res. 408: 399- 402.

Hjertan S and Pan H. (1983) Purification and characterization of two forms of a low affinity Ca^{2+}-ATPase from erythrocyte membranes. Biochem Biophys Acta 728: 281-288.

Hong J, Bose M, Ju J, Ryu hm J, Chenm X, Sang S, Lee MJ and Yang CS. (2004) Modulation of arachidonic acid metabolism by curcumin and related β-diketone derivatives: effects on cytosolic phospholipase A(2), cyclooxygenases and 5-lipoxygenase. Carcinogenesis 25: 1671-1679.

Jenner P. (1998) Oxidative mechanisms in nigral cell death in Parkinson's disease. Movement Disord. 1: 24-34.

Jiang ZY, Hunt JV and Wolff SD. (1992) Ferrous sulphate oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low-density lipoprotein. Analytical Biochem. 202: 384-389.

Joseph P, Muchnok TK, Klishis ML, Roberts JR, Antonini JM, Whong WZ and Ong T. (2001) Cadmium induced cell transformation and tumourogenesis are associated with transcriptional activation of c-fos, c-jun, and c-myc protooncogenes: role of cellular calcium and reactive oxygen species. Toxicol Sci. 61: 295-303.

Kakkar P, Das B and Viswanathan PN. (1984) A modified spectroscopic assay of superoxide dismutase. Indian J Med Res. 21: 130-132.

Kasote DM, Hegde MV and Katyare SS. (2013) Mitochondrial dysfunction in psychiatric and neurological diseases: cause(s), consequence(s), and implications of antioxidant therapy. BioFactors 39: 392-406.

Keifer Matthew C and Firestone J. (2007) Neurotoxicity of pesticides. J Agromed. 12(1): 17-25.

Kilburn Kaye H. (2004) Role of molds and mycotoxins in being sick in buildings: Neurobehavioral and pulmonary impairment. Adv Appl Microbiol. 55: 339-359.

Kovac S, Kostova ATD. Herrmann AM, Melzer N, Meuth SG and Gorji A. (2017) Metabolic and homeostatic changes in seizures and acquired epilepsy—mitochondria, calcium dynamics and reactive oxygen species. Int J Mol Sci. 18: 1935.

Levine RL, Garland D, Oliver CN, Amic A, Climent I, Lenz AG, Ahn BW, Shaltiel S and Stadtman ER. (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 186: 464-478.

Lin JK and Lin–Shiau SY. Mechanisms of cancer chemoprevention by curcumin. Proc Nat Sci Council Republic China 25: 59-66.

Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ. (1951) Protein measurment with Folins Phenol reagent. J Biol Chem. 193: 265-278.

Lv C, Maharjan S, Wang Q, Sun Y, Han X, Wang S, Mao Z, Xin Y and Zhang B. (2017) α-Lipoic acid promotes neurological recovery after ischemic stroke by activating the Nrf2/HO-1 pathway to attenuate oxidative damage. Cell Physiol Biochem. 43: 1273–1287.

Manca D, Ricard AC, Trottier B and Chevalier G. (1991) Studies on lipid peroxidation in rat tissues following administration of lowand moderate doses of cadmium chloride. Toxicology 67: 303-323.

Mao XY, Zhou HH and Jin WL. (2019) Redox-related neuronal death and crosstalk as drug targets: Focus on epilepsy. Front Neurosci. 13: 512.

Mata M, Fink DJ, Gainer H, Smith CB, Davidsen L, Savakis H, Schwartz WJ and Sokoloff L. (1980) Activity-dependent energy metabolism in rat posterior pituitary, primarily reflects sodium pump activity. J Neurochem. 34: 214-215.

McCoy CR, Sabbagh MN, Huaman JP, Pickrell AM and Clinton SM. (2019) Oxidative metabolism alterations in the emotional brain of anxiety-prone rats. Prog Neuropsychopharmacol Biol Psychiatry 95: 109706.

Morelli MB, Gambardella J, Castellanos V, Trimarco V and Santulli G. (2020) Vitamin C and cardiovascular disease: An update. Antioxidants 9: 1227.

Moron MS, Despierre JW and Minnervik B. (1979) Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochim Biophys Acta 582: 67-78.

Murugan P. (2023) A review on antidiabetic effect of tetrahydrocurcumin in type 2 diabetes. J Adv Zool. 44 (S-7): 147-156.

Murugan P. (2010) Taner's Cassia (*Cassia auriculata* L) extract prevents hemoglobin glycation tail tendon collagen properties in experimental diabetic rats. J Cell Tissue Res. 10(1): 2109-2114.

Murugan P. (2015 a) Preventive effects of *Cassia auriculata* on brain lipid peroxidation streptozotocin diabetic rats. Int J Information Res Rev 2(5): 6924-6929.

Murugan P. (2015 b) Effect of *Cassia auriculata* L on erythrocyte membrane bound enzymes andantioxidant status in experimental diabetes. Int J Rec Adv Multidiscipl Res. 2(12): 5760-5764.

Murugan P. (2015 c) Effect of *Cassia auriculata* L on plasma antioxidants in streptozotocin-nicotinamide induced experimental diabetes. Int J Information Res Rev. 2(5): 6930-6934.

Murugan P. (2015 d) A review on some phytochemicals on diabetes. Int J Curr Res Life Sci. 4(1): 250-253.

Murugan P. (2021 a) Antioxidants effect on herbs. Int J Curr Res Life Sci. 10(1): 3399-3402.

Murugan P. (2021 b) Effect of tetrahydrocurcumin on lipid profiles in streptozotocin–nicotinamide induced type 2 diabetes mellitus. Plant Arch. 1: 1265-1269.

Murugan P. (2021 c) Antidiabetic effect on some medicinal plants. Int J Curr Res Life Sci. 10(1): 3392-3395.

Murugan P. (2021 d) A review of drug-induced liver injury. Adv Pharmacol Toxicol. 22(1): 1-9.

Murugan P. (2021 e) Hepatoprotective effect on medicinal plants. Int J Curr Res Life Sci. 10(1): 3396-3398.

Murugan P. (2022) Antioxidant effect of tetrahydrocurcumin compared pterostilbene in streptozotocin - nicotinamide induced diabetic rats. J Pharmaceut Negative Results 13(4): 2190-2196.

Murugan P. (2023 a) Effect of tetrahydrocurcumin and pterostilbene on lipid peroxidation and lipids in streptozotocin - nicotinamide induced diabetic rats. J Population Therapeut Clin Pharmacol. 30(4): 649-655.

Murugan P. (2023 b) Effect of tetrahydrocurcumin and pterostilbene on changes in glycoprotein components in streptozotocin induced diabetes. Eurpoan Chem Bull. 12(10): 2749-2766.

Murugan P. (2023 c) Antihyperglycaemic effect of tetrahydrocurcumin and pterostilbene: effect on key metabolic enzymes of carbohydrate metabolism in streptozotocin induced diabetes. J Adv Zool. 44 (S5): 2271-2279.

Murugan P and Maneemegalai S. (2024) Modulatory effect of pterostilbene on tail tendon collagen in type 2 diabetic rats. Chelonian Conserv Biol. 19(1):111-125.

Murugan P and Pari L. (2005) Effect of tetrahydrocurcumin on erythromycin estolate-induced lipid peroxidation in rats. J Basic Clin Physiol Pharmacol. 16: 1-15.

Murugan P and Sakthivel V. (2021) Effect of pterostilbene compared to tetrahydrocurcumin on liver and kidney functional markers and protein levels in experimental diabetes. J Pharmaceut Negative Results 12(1): 103-109.

Naito M, Wu X, Normura H, Kodama M, Kato Y and Osaswa T. (2002) The protective effect of tetrahydrocurcumin on oxidative stress in

cholesterol-fed rabbits. J Atherosclerosis Thrombosis 9: 243-250.

Niehaus WG and Samuelsson D. (1968) Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem. 6: 126-130.

Pari L and Murugan P. (2004) Protective role of tetrahydrocurcumin against erythromycin estolate induced hepatotoxicity. Pharmaco Res. 49(5): 481-486.

Pateron D, Vicaut E, Debuc E, Sahraoui K, Carbonell N, Bobbia X, Thabut D, Adnet F, Nahon P, Amathieu R, Aout M and Javaud N. (2011) Erythromycin infusion or gastric lavage for upper gastrointestinal bleeding: a multicenter randomized controlled trial. Ann Emerg Med. 57(6): 582-589.

Ritchie Glenn D, Still Kenneth R, Alexander William K, Nordholm Alan F, Wilson Cody L, Rossi John and Mattie David R. (2001) A review of the neurotoxicity risk of selected hydrocarbon fuels. J Toxicol Environ Hlth Part B Crit Rev. 4 (3): 223-312.

Rodrigo R, Fernandez-Gajardo R, Gutierrez R, Matamala J, Carrasco R, Miranda-Merchak A and Feuerhake W. (2013) Oxidative stress and pathophysiology of ischemic stroke: Novel therapeutic opportunities. CNS Neurol Disord Drug Targets 12: 698-714.

Rotruck JT, Pope AL and Ganther HE. (1973) Selenium biochemical role as a component of glutathione peroxidase purification assay. Science 179: 588-590.

Sainio MA Sr. (2015) Neurotoxicity of solvents. Handb Clin Neurol. 131: 93-110.

Sajithlal GB, Chithra P and Gowri C. (1998) Effect of curcumin on the advanced glycation and cross-linking of collagen in diabetic rats. Biochem Pharmacol. 56: 1607-1614.

Sanui H and Rubin H. (1982) The role of magnesium in cell proliferation and transformation. In: Ions, Cell Proliferation and Cancer, (eds.) Boynton A.L., McKochan, W.L., and Whitfield, J.P., Academic Press, New York, pp. 517-537.

Schulz JB, Lindenau J, Seyfried J and Dichgans J. (2000) Glutathione oxidative stress and neurodegeneration. Eur J Biochem. 267: 4904-4911.

Shinto L, Quinn J, Montine T, Dodge HH, Woodward W, Baldauf-Wagner S, Waichunas D, Bumgarner L, Bourdette D and Silbert L. (2014) A randomized placebo-controlled pilot trial of omega-3 fatty acids and alpha lipoic acid in Alzheimer's disease. J Alzheimer's Dis. 38: 111-120.

Sinha AK. (1972) Colorimetric assay of catalase. Analytical Biochem. 47: 389-394.

Slyshenkov VS, Shevalye AA, Liopo AV and Wojtczak L. (2002) Protective role of l-methionine against free radical damage of rat brain synaptosomes. Acta Biochim. 4: 907-916.

Snavely S and Hodges G. (1984) The neurotoxicity of antibacterial agents. Ann Intern Med. 101: 92-104.

Szabó C, Ischiropoulos H and Radi R. (2007) Peroxynitrite: Biochemistry, pathophysiology and development of therapeutics. Nat Rev Drug Discov. 6: 662-680.

Taghizadeh M, Tamtaji OR, Dadgostar E, Daneshvar Kakhaki R, Bahmani F, Abolhassani J, Aarabi MH, Kouchaki E, Memarzadeh MR and Asemi Z. (2017) The effects of omega-3 fatty acids and vitamin E co-supplementation on clinical and metabolic status in patients with Parkinson's disease: A randomized, double-blind, placebo-controlled trial. Neurochem Int. 108: 183-189.

Thijs RD, Surges R, O'Brien TJ and Sander JW. (2019) Epilepsy in Adults. Lancet 393: 689-701.

Yang DQ, Zuo QN, Wang T, Xu D, Lian L, Gao LJ, Wan C, Chen L, Wen FQ and Shen YC. (2021) Mitochondrial-targeting antioxidant SS-31 suppresses airway inflammation and oxidative stress induced by cigarette smoke. Oxid Med Cell Longev. 2021: 6644238.