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Neuroprotective role of antioxidant and pyranocarboxylic acid derivative against AlCl₃ induced Alzheimer's disease in rats

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PEER REVIEW

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Comments

This is a valuable research work in which authors have demonstrated the neuroprotective effect of quercetin and etodolac in combination against aluminium chloride induced AD in rats. In the present study, chronic administration of drugs in combination attenuated the cognitive dysfunction and decreased oxidative stress markers more effectively rather than individual drugs which have been proved from the biochemical and memory test assessment.

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ABSTRACT

Objective: To assess potential of quercetin and etodolac to treat oxidative stress in neuronal death and inflammation in Alzheimer's disease of AlCl₃ induced rat models. All results of this AlCl₃ model are compared with those obtained in controls.

Methods: Wistar rats, housed in a controlled environment were treated with aluminum chloride (4.2 mg/kg of body weight, *i.p.*) for 28 d rather than oral to ensure neurotoxic concentration in hippocampus and hypothalamic region, part highly active in memory control and cognition, while control group was injected with saline. Estimation of thiobarbituric acid reactive substance, superoxide dismutase, reduced glutathione and acetylcholine levels gave estimation of neuronal damage. Low (20 mg/kg and 25 mg/kg) and high (40 mg/kg and 50 mg/kg) doses of quercetin and etodolac were administered to the test groups respectively. Histopathology study was conducted to perform relative study.

Results: Co-administration of quercetin and etodolac either alone or in combination prevented the changes in biochemical markers of Alzheimer's disease, but significant results ($P < 0.05$) were seen when a combination of two was administered at low dose levels. Good correlation was developed between chemical estimations and histopathology study.

Conclusions: Our findings suggest a combined role of anti-oxidant and cyclooxygenase inhibitor in protection of neural degeneration and inflammation due to oxidative stress.

KEYWORDS

Quercetin, Etodolac, Aluminum chloride, Superoxide dismutase, Reduced glutathione, Thiobarbituric acid reactive substance

1. Introduction

Alzheimer's disease (AD) is a fatal neurodegenerative disorder manifested by cognitive and memory deterioration, behavioral disturbances impairment in daily living activities and neuropsychiatric symptoms. According to free radical hypothesis, aging process is associated with multisystem

failure due to oxidative damage caused by imbalance between reactive oxygen species production and antioxidant defenses^[1,2]. The cytopathological significances of oxidative damage is supported by findings of upregulation of antioxidant enzymes like hemeoxygenase–1 and superoxide dismutase in neurons of AD^[3–5].

Furthermore lipid peroxidation (LPO), a hallmark of

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oxidative tissue injury has been found to be elevated in AD brain[6]. Free radical mediated LPO has been shown to activate cyclooxygenase (COX-2). Furthermore, the two step oxygenase and peroxidase action of COX leading to the formation of reactive oxygen species and prostaglandin H₂. Aggregated synthetic A β 1–40 peptides have been shown to induce COX-2 expression in SH-SY5Y neuroblastoma cells, and A β 1–40 has been shown to stimulate COX-2 oxygenase and peroxidase activity in a cell free system; these findings were further supported by evidences showing that increased basal levels of oxidative stress significantly increases A β neurotoxicity in hippocampal neurons *in-vitro*. It was reported that patients of arthritis undergoing nonsteroidal anti-inflammatory drug (NSAID) therapy have reduced risk of developing AD. New evidence that COX is involved in neurodegeneration has led to renewed interest in the therapeutic activity of NSAIDs in AD. Aluminium is a constituent of antacids, deodorants and food additives which allowed easy access into the body. Aluminium neurotoxicity in animals has been clearly established and shown to be involved in etiology of neurodegenerative diseases such as AD, amyotrophic amyotrophic lateral sclerosis, Guam-Parkinson's dementia *etc.* Aluminium promotes the formation of amyloid- β protein plaques by aggregating tau proteins in AD. It has also been implicated in aging related changes and neurodegenerative diseases. It is reported that aluminium toxicity is due to potentiation of activity of Fe²⁺ and Fe³⁺ ions at to cause oxidative damage. It also interacts with calcium binding sites and disrupts calcium homeostasis and thereby induces neurodegeneration. Aluminium toxicity was found to be associated with reduced axonal length and dendritic branches in hippocampus[7–23].

Flavonoids are polyphenolic compounds found in vegetable food. Quercetin (3,5,7,3',4' pentahydroxy flavon) is a member of the flavonoid family and its effect are including anti cancerogenic, antiviral, antithrombotic, anti-ischemic, anti-inflammatory and anti-allergic activity as well as preventive influence in atherosclerosis and coronary heart disease[24–26]. Quercetin has been shown to scavenge O₂, singlet oxygen (¹O₂) and OH radicals to prevent LPO to inhibit cyclooxygenase and lipoxygenase enzymes and to chelate transition meta ion such as Fe and Cu[27–29]. It has been reported that quercetin may provide a promising approach for the treatment of AD and other oxidative-stress related neurodegenerative diseases. It is not only as oxidant but also as pro-oxidant. Etodolac pyranocarboxylic acid derivative (NSAID) nonsteroidal anti-inflammatory drug have been used for the treatment of arthritis for the past decade without much significant cardiac effects and other COX-2 specific side effects because sparing of PGE₂ production

by etodolac[30–32]. So our study is aimed at examination of neuroprotective effect of quercetin and etodolac in combination against aluminium chloride (AlCl₃) induced AD in rats.

2. Materials and methods

2.1. Materials

AlCl₃ was obtained from Loba chemical. Etodolac was a gift sample from Ranbaxy Pharmaceuticals Poanta Sahib. Quercetin was purchased from Hi-Media. 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), heparin and acetylthiocholine were purchased from Sigma Chemicals Company.

2.2. Animals

Sprague-Dawley rats of either sex, with an average weight of (200±30) g were used in this study. Animals were housed in polypropylene cages with dust free rice husk with control environment of temperature (23±2) °C, humidity (40%–60%), sound proof room and 12:12 h light dark cycles as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) norms guidelines India. They were provided with free access of standard rodent chow/feed and water *ad libitum*. The experimental procedure was approved by the Institutional Animal Ethics Committee of ASBASJSMCOP Bela (Ropar) Punjab and from CPCSEA prior to initiation of experiment.

2.3. Dosage regimen

Animals were divided into seven treatment groups. Group I: vehicle control group (normal saline used as vehicle for all the drug as well as aluminium); Group II: disease control group (AlCl₃) (4.2 mg/kg, *i.p.*, daily for 28 d); Group III: quercetin (low dose) drug treatment (25 mg/kg per day *i.p.* body weight); Group IV: quercetin (high dose) (50 mg/kg per day *i.p.* body weight); Group V: etodolac (low dose) (20 mg/kg per day *i.p.* body weight); Group six: etodolac (high dose) (40 mg/kg per day *i.p.* body weight); Group VII: combination of quercetin (low dose 25 mg/kg per day *i.p.*) and etodolac (20 mg/kg per day *i.p.*).

2.4. In vivo treatment

Animals in all groups were treated for 28 d as per above treatment. Animals were sacrificed by decapitation. Prior

to decapitation, blood was collected from retro-orbital on Day 29 and the intact brain was washed with saline to clean traces of blood. The brain thus collected was processed further for acetylcholinesterase (AChE) estimation, LPO estimation, whereas glutathione estimation (GSH), superoxide dismutase (SOD) activity were assayed in the blood collected.

2.5. Assay of memory

The step-down test apparatus was made according to previous reports^[33,34]. Briefly, rats were placed in 25 cm×22 cm×30 cm acrylic training apparatus in which the floor is a series of parallel bronze bars spaced 0.8 cm apart. A platform was placed at the left corner of the box at the height of 5 cm. To test the learning ability and to train the rats, 3 min after placing rats on the floor of the box for adaptation, rats received a 36 V foot shock. Most rats jumped onto the platform located within the training apparatus. Rats that did not respond to electric shock and jump onto the platform were not used in the experiments. Rats on the platform would stay on the platform in a short time and jump back to the floor where another electric shock was applied. This training lasted 5 min. We did not observe a difference in learning among groups of rats. Memory retention test was evaluated 24 h post-training. To do so, each rat was placed on the platform. The time that each rat spent on the platform before jumping off the platform to the grid on the first time was recorded as latency time. The number of times of each rat jumping off the platform to the grid and receiving electric shock during a period of 5 min were recorded as the number of mistake the rat made. The time of latency and the number of mistake are indicators of memory ability.

2.6. Biochemical estimation

To carry out various estimations, brains were removed immediately rinsed in ice cold normal saline and blotted dry. Tissue was homogenized with ice cold 10 mM phosphate buffer saline (pH 7.4). Homogenate was centrifuged at 2000 r/min for 10 min at 4 °C to remove the cell debris and nuclear pellet.

2.6.1. Acetylcholinesterase activity

Acetylcholine (ACh) has a very short half-life and direct estimation of ACh is a little difficult in brain homogenates. There are several approaches to evaluate cholinergic function indirectly.

Estimation of AChE activity provides a relatively easy and valuable assessment of cholinergic function. The

method of AChE activity estimation is popularly known as Ellman's method named after George Ellman who developed this method in 1961. The esterase activity was measured by providing an artificial substrate, acetylthiocholine. Because of the cleavage of acetylthiocholine by AChE is allowed to react with the -SH reagent DTNB, which is reduced to thionitrobenzoic acid, a yellow colored anion with an absorption maxima at 412 nm. The concentration of thionitrobenzoic acid detected using a UV spectrophotometer was then taken as a direct estimate of the AChE activity.

2.6.2. LPO

Animals were sacrificed and brain was removed and homogenized in phosphate buffer (pH 7.4, 10% w/v). The clear supernatant, obtained after centrifugation at 3000 r/min for 15 min was used to calculate thiobarbituric acid reactive substance (TBARS).

The quantitative measurement of TBARS, an index of LPO in brain was performed according to the method of Ohkawa *et al*^[35]. About 0.2 mL of supernatant of brain homogenate was pipette out in a test tube, followed by addition of 0.2 mL of 8.1% dodecyl sulphate, 1.5 mL of 30% acetic acid (pH 3.5), 1.5 of 0.8% of thio-barbituric acid and the volume was made up to 4 mL with distilled water. The test tubes were incubated for 1 h at 95 °C, then cooled and added 1 mL of distilled water followed by addition of 5 mL of *n*-butanol-pyridine mixture (15:1 v/v). The tubes were centrifuged at 4000 r/min for 10 min. The absorbance of developed pink colour was measured spectrophotometrically at 532 nm. A standard calibration curve was prepared using 1-10 nmol/L of 1,1,3,3 tetra methoxy propane. The TBARS value was expressed as nanomoles per mg of protein.

2.6.3. Superoxide dismutase

Superoxide dismutase activity was measured according to the method described by Marklund S and Marklund G^[36]. The method is based on ability of SOD to inhibit the auto oxidation of pyrogallol at alkaline pH (8.2). Enzyme activity was expressed as units/mg protein.

2.6.4. Determination of circulating superoxide dismutase levels

On Day 29 of treatment, blood was collected from the rats from retro-orbital in heparinized disposable syringes. The blood was then centrifuged at 4000 r/min for 7 min at 4 °C, and the plasma was separated and assayed for SOD.

The remaining red blood cells (RBCs) were then subjected for extraction of superoxide dismutase. Heparinized blood was centrifuged at 2500 r/min for 30 min at 4 °C and the

plasma was carefully separated. Five milliliters of normal saline (0.9%) were added to the erythrocyte pellet, mixed, and the resuspension was transferred to 15 mL centrifuge tubes. After the erythrocytes were washed three times with saline as above, they were diluted with 4 mL double distilled water in order to lyse them. One milliliter of ethyl alcohol and 0.6 mL of chloroform were added to separate the hemoglobin. The tubes were shaken vigorously for 15 min and centrifuged at 2500 r/min for 10 min at 4 °C. The water–ethanol layer was aspirated and diluted with 0.7 mL of double distilled water. SOD activity was measured in the hydro–alcoholic layer.

2.6.5. GSH estimation

GSH estimation was done according to the method Ellman 1959 using DTNB water soluble compound for quantitating free sulfhydryl groups in solution. It produces yellow colored product when it reacts with sulfhydryl. Plot a standard curve by preparing a set of dilution of known concentration with cysteine hydrochloride. Measure the absorbance of unknown samples at 412 nm. Total protein estimation was estimated in the samples by method Lowry *et al*[37].

2.7. Statistical analysis

Data were expressed as mean±SD. Statistical comparisons were performed by One–way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test. The level of significance was accepted at $P<0.05$.

3. Results

3.1. Biochemical analysis

Tables 1 and 2 showed that $AlCl_3$ treatment for 28 d at a dose of 4.2 mg/kg per day of body weight by *i.p.* route resulted in severe alterations in oxidative stress marker parameters as well as AchE levels. Significant decrease was observed in AchE levels and an increase in LPO levels. Improvement was observed with the treatment of therapeutic agents. However greater beneficial effects were observed when combination of two therapeutic agents (etodolac and quercetin) was given.

Table 3 shows the effect of quercetin and etodolac either alone or in combination against the $AlCl_3$ –mediated toxicity. Significant results were seen with the treatment of combination of drugs at lower dose level.

Figure 1 shows changes in different cellular structure of

hippocampal cells in different groups.

Table 1

Effects of aluminium chloride, quercetin and etodolac on LPO levels and AchE activity.

Groups	LPO (nmol/g of protein)	AchE (moles of substrate hydrolysed/min/g tissue)
Group I (Control)	6.230 00±0.004 40 ^a	7.294 00±0.089 79 ^a
Group II ($AlCl_3$ treated)	18.700 00±0.011 50 ^b	1.872 00±0.113 30 ^b
Group III	8.340 00±0.003 51 ^b	4.009 00±0.155 90 ^b
Group IV	7.330 00±0.004 19 ^b	5.402 00±0.126 50 ^b
Group V	9.350 00±0.008 54 ^b	3.333 00±0.113 10 ^b
Group VI	10.300 00±0.007 48 ^b	4.613 00±0.098 20 ^b
Group VII	7.430 00±0.005 98 ^b	6.085 00±0.081 40 ^b

Results were expressed as mean±SEM. Aluminium treated group was compared to the control group and drug treated groups were compared with aluminium treated group. ^aLevel of significance ($P<0.05$) in comparison to Group I (untreated control rats). ^bLevels of significance ($P<0.05$) in comparison to Group II (aluminium chloride treated rats).

Table 2

Effect of Aluminium chloride, quercetin and etodolac on antioxidant enzymes SOD, GSH.

Groups	SOD in RBC (units/mg protein)	SOD in plasma (units/mg of protein)	GSH (nmol/L DTNB oxidized/min/g tissue)
Group I	35.150 0±0.312 8	37.700±0.477	0.461 80±0.001 30
Group II	27.390 0±0.305 7 ^a	29.700±0.337 ^a	0.283 20±0.007 20 ^a
Group III	36.230 0±0.267 6 ^b	38.700±0.397 ^b	0.381 40±0.000 90 ^b
Group IV	38.520 0±0.282 0 ^b	39.900±0.479 ^b	0.426 80±0.001 10 ^b
Group V	32.500 0±0.273 5 ^b	34.600±0.425 ^b	0.361 40±0.001 00 ^b
Group VI	33.520 0±0.462 7 ^b	35.100±0.335 ^b	0.405 60±0.001 80 ^b
Group VII	37.660 0±0.191 7 ^b	39.800±0.426 ^b	0.435 00±0.005 30 ^b

Results were expressed as mean±SEM. Aluminium treated group was compared to the control group and drug treated groups were compared with aluminium treated group. ^aLevel of significance ($P<0.05$) in comparison to Group I (untreated control rats). ^bLevels of significance ($P<0.05$) in comparison to Group II (aluminium chloride treated rats).

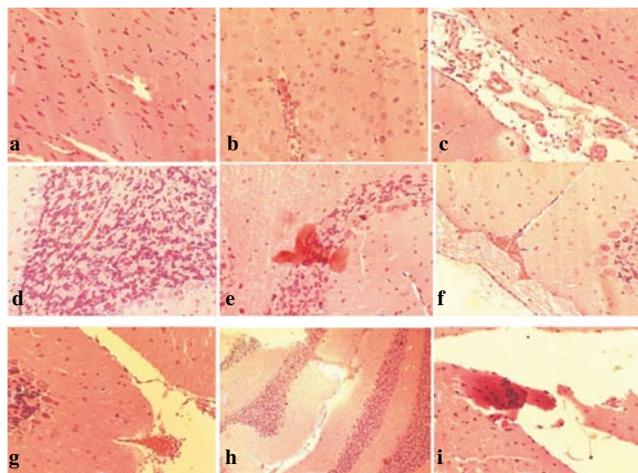


Figure 1. Changes in different cellular structure of hippocampal cells in different groups (40X).

a: Control group, b & c: $AlCl_3$ treated groups, d & e: Quercetin treated groups, f & g: Etodolac treated groups, h & i: Combination treated groups.

3.2. Behavioral analysis

Step down apparatus latency test show the average latency of animal to step down on the grill by different experimental rats. Our observation revealed that all experimental groups learned to escape electric shock by finding the wooden platform in

Table 3

Effect of drugs Quercetin and Etodolac either alone or in combination on step down latency in rats.

Group	Treatment	Dose (mg/kg body weight per day)	Latency time (seconds)			
			Week 1	Week 2	Week 3	Week 4
I	Normal saline	0.5 mL	300.000±0.000	300.000±0.000	299.800±0.200 [#]	300.000±0.000 ^{**}
II	Aluminium chloride	4.2	299.200±0.800	295.000±2.498	289.600±1.435 [#]	274.800±1.715 ^{###}
III	Quercetin+Aluminium chloride	25+4.2	299.600±0.400	295.000±2.739	295.200±1.281	287.000±1.643 ^{***}
IV	Quercetin+Aluminium chloride	50+4.2	300.000±0.000	294.600±3.736	292.600±2.182	287.600±1.077 [*]
V	Etodolac+Aluminium chloride	20+4.2	300.000±0.000	296.400±1.691	292.800±1.241	288.200±0.583 ^{**}
VI	Etodolac+Aluminium chloride	40+4.2	300.000±0.000	296.400±1.503	292.800±2.177	287.400±1.364 ^{***}
VII	Quercetin+Etodolac+Aluminium chloride	25+20+4.2	300.000±0.000	297.800±1.020	296.200±1.241 [*]	293.800±1.530 ^{***}

Data are expressed as mean value±SEM, n=5. [#]P<0.001, ^{##}P<0.0001 when compared control group with positive control (aluminium chloride) (using unpaired student's t test). ^{*}P<0.05, ^{**}P<0.01, ^{***}P<0.001 when compared with positive control (aluminium chloride) ANOVA followed by dunett's multiple comparison test.

3 experimental days. Results of step down test confirmed a significant effect of aluminium toxicity as well as of drugs either alone or in combination as depicted in the Table 3.

4. Discussion

Trace element neurotoxicity has been hypothesized to play a role in AD for many years. Numerous elements have been reported to be imbalanced in AD. The elements receiving the most attention in AD are aluminum (Al), mercury (Hg), zinc (Zn), and iron (Fe). Emphasis is usually placed on elevated concentrations of elements in AD, with the suggestion that they have a direct toxic effect on neurons[38]. It is now well reported that *i.p.* administration of aluminum chloride induced cognitive dysfunction in time dependent manner and its toxicity is thought to be one of the causative agents of AD[39]. Oral administration of AlCl₃ resulted in a significant increase of metal concentration in ventral, midbrain while *i.p.* administration led to decrease in metal concentration of cerebral regions. Administration of AlCl₃ (*i.p.*) predominantly accumulates in the hippocampus and this region is known to be particularly susceptible in AD and has important role in learning and memory functions[23]. For this reason AlCl₃ model was selected for our study.

Brain cell possesses transferrin receptor, a high affinity and specific receptor for transferrin which binds to aluminium primarily bounded to serum iron transport protein, transferrin. Aluminium coordinated neuronal membrane facilitating attacks by iron induced free radicals whereas membrane oxidation in turn increases aluminium binding, thereby aggravating oxidation[40]. The most important well established fact is the definite evidence that aluminium can effectively aggravate iron initiated LPO. Transferrin receptor is postulated to be an access point for iron into brain from circulation. Binding of aluminium to transferring reduces binding of iron to protein resulting in an increased concentration of free iron in intracellular spaces. The previous studies have documented that this free iron can cause peroxidation of membrane lipids resulting in memory damage[35]. It has been reported that AlCl₃ induces the generation of free radical formation, which

further induces LPO which activates cyclooxygenase COX-2 leading to the formation of a reactive oxygen species and prostaglandin H₂ (PGH₂)[9]. In present study, the lipid damage was measured in form of TBA-RS. The increased accumulation of these products is strongly reflected by AlCl₃ inflicted oxidative damage. TBA-RS is the secondary byproduct of LPO and has shown to catalyze the process of oxidative insult to membrane, so here LPO was found to be significantly increased with AlCl₃ exposure (4.2 mg/kg per day *i.p.* for 28 d) 33.68% with respect to normal saline. The observations are in accordance to previous studies which have shown that the mechanism of action of COX-2 inhibitor is believed to include inhibition of prostaglandin synthesis via selective inhibition of COX-2. In our study, the levels of LPO were significantly decreased in etodolac low dose and high dose treatment groups respectively. Whereas, quercetin at the dose level of 25 and 50 mg/kg per day treatment decreased the LPO levels. Furthermore, quercetin is known as excellent metal chelators. It was confirmed that both antiradical and chelating effects were involved in the protective effect of quercetin, a possible mechanism of neuroprotection. On combination treatment with COX-2 inhibitor (etodolac) and antioxidant drug (quercetin), LPO levels were significantly decreased by 39.73% as compared to groups treated with individual drugs.

Aluminium intoxicated animals showed a number of indicators of oxidative stress. This included increases in the levels of TBARS and decreases in the levels of GSH and SOD. GSH is a major low molecular weight thiol compound of living cells and plays an important role in the detoxification of potentially toxic compounds by its conjunction with xenobiotics or their metabolites[41]. In the present study, animals exposed to AlCl₃ registered a decrease in the levels of GSH in rat brains by 49.13%. The level of GSH is maintained by reduction of oxidized GSH by NADPH via GSH reductase and by *de novo* synthesis from glutamic acid, cysteine and glycine. GSH reductase catalyzes the reaction that converts oxidized GSH to reduced GSH. This reaction requires NADPH as a source of reducing power which is generated by the hexose monophosphate pathway[42]. Anti-oxidant (quercetin) has shown increase in the levels of GSH by 21.26% and 31.09% at 25 and 50 mg/kg per day. Quercetin has been

shown to down regulate the NADPH which is a source of reducing power. Treatment of group with COX-2 inhibitor previously treated with AlCl₃ have shown increase in the levels of GSH by 16% and 25% at 20 and 40 mg/kg per day dose respectively whereas significant increase in the levels by 32% were observed with combination therapy of COX-2 and anti-oxidant. It has been reported that AlCl₃ induces the generation of free radical formation, which further induces LPO which activates cyclooxygenase COX-2 leading to the formation of a reactive oxygen species and prostaglandin H₂ (PGH₂)^[9].

Natural anti-oxidant enzymes like SOD, glutathione peroxidase, and glutathione S-transferase present the first line of defense against free radical damage under oxidative stress conditions. In current study, we observed that with AlCl₃ administration (4.2 mg/kg per day *i.p.* for 28 d) the activity of anti-oxidant enzyme (SOD) was significantly decreased by 21.22% as compared to control. The decreased activity might have resulted from oxidative modification of proteins. In AlCl₃ treated rats, the lower activity of natural oxidants resulted in a decrease of anti-oxidant versus oxidant ratio. Usually the decreased ratio (anti-oxidant:oxidant) plays a crucial role in generating a condition of oxidative stress^[43]. Post-treatment with COX-2 and anti-oxidant individually increased the level of SOD in plasma as well as in RBC. However significant elevation was observed when animals were treated with a combination of COX-2 inhibitor and anti-oxidant.

To validate potential of drugs interceptive model was employed. AlCl₃ (*i.p.*) induced significant memory impairment in step down apparatus at later time points perceived by latency time and number of mistakes done by animal under trial, which was attenuated with quercetin and etodolac either alone or in combination, but the most significant results are seen with combination of both drugs etodolac and quercetin in low dose combination revealing therapeutic potential of combination. Increase in latency time and decrease in number of mistakes in behavioral model on chronic administration of drugs might have attenuated the cognitive dysfunction and decreased oxidative stress depicted from the levels of oxidative stress markers. It is well established that COX-2 is implicated in neurodegenerative processes and expression of COX-2 enzyme is up regulated in cortical and hippocampal pyramidal circuits, the structure associated with memory and learning in AD contributing to behavioral and pathological changes associated with AD^[9]. Moreover bilateral intrahippocampal injection of PGE₂, endotoxin or iL-1 β , potent inducer of COX-2 significantly impaired memory that resembles AD which was attenuated by pretreatment with selective COX-2 or nonselective COX-1 inhibitor.

Several studies demonstrate that chronic stress leads to cognitive dysfunction and results in disorders. Studies on brains from patient suffering from AD have shown reduced AchE activity in the hippocampus and cortex^[44].

Evidence on role of AchE in cognitive function also comes from studies in biopsy tissues of AD patients, which show decreased AchE activity with concurrent loss of cognitive function^[45]. Linking the involvement of the impairment of cellular oxidant anti-oxidant defense resulted in generation of free radical, decreased in cholinergic activity and also cognitive dysfunction following intra-peritoneal injection of AlCl₃. AchE is decreased following long term postnatal exposure of AlCl₃^[39]. Our study showed that administration of AlCl₃ (4.2 mg/kg per day *i.p.* for 28 d) is concurrent with decreased AchE activity^[46]. Animals exposed to aluminium registered decrease in levels of AchE as compared to normal saline. Post treatment with anti-oxidant, quercetin increased the AchE activity. While on treatment with COX-2 inhibitor, the AchE activity was found to be increased. But a significant increase in levels of AchE activity was registered on administration of combination of anti-oxidant (quercetin) and COX-2 inhibitor (etodolac). Findings from behavioral memory assessment test are highly concurrent with the variation in the levels of AchE observed. In the present study, chronic administration of drugs in combination attenuated the cognitive dysfunction and decreased oxidative stress markers more effectively rather than individual drugs which have been proved from the biochemical and memory test assessment.

We concluded that chronic treatment with NSAID etodolac (pyranocarboxylic acid derivative) in combination with antioxidant (quercetin), resulted in significant improvement in memory impairment, prevented increased LPO levels and depletion of GSH, restored the decreased levels of super oxide dismutase both in plasma and RBC, restored the decreased activity of acetylcholine and decreased the number of mistakes and increased the latency time of rats in the behavioral model of step down apparatus.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Flavonoids are polyphenolic compounds found in

vegetable food. Quercetin, a member of the flavonoid family, contains anticarcinogenic, antiviral, antithrombotic, anti-ischemic, anti-inflammatory and anti-allergic activity as well as preventive influence in atherosclerosis and coronary heart disease. It has been reported that quercetin may provide a promising approach for the treatment of AD and other oxidative-stress related neurodegenerative diseases. It is not only as oxidant but also as pro-oxidant. Etodolac NSAID has been used for the treatment of arthritis for the past decade without much significant cardiac effects and other COX-2 specific side effects because sparing of PGE2 production by etodolac.

Research frontiers

This study is aimed at examination of neuroprotective effect of quercetin and etodolac in combination against aluminium chloride induced AD in rats.

Related reports

It has been reported that quercetin may provide a promising approach for the treatment of AD and other oxidative-stress related neurodegenerative diseases.

Innovations and breakthroughs

In the present study, chronic administration of drugs in combination attenuated the cognitive dysfunction and decreased oxidative stress markers more effectively rather than individual drugs which have been proved from the biochemical and memory test assessment.

Applications

Result of the article suggests that chronic treatment with NSAID etodolac (pyranocarboxylic acid derivative) in combination with antioxidant (quercetin), may result in significant improvement in memory impairment and can prevent increased LPO levels and depletion of GSH. It can restore the decreased levels of super oxide dismutase both in plasma and RBC as well as the decreased activity of acetylcholine.

Peer review

This is a valuable research work in which authors have demonstrated the neuroprotective effect of quercetin and etodolac in combination against aluminium chloride induced AD in rats. In the present study, chronic administration of drugs in combination attenuated the cognitive dysfunction and decreased oxidative stress markers more effectively rather than individual drugs which have been proved from the biochemical and memory test assessment.

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