Current Research in Pharmaceutical Sciences
Available online at www.crpsonline.com

ISSN: 2250 – 2688

Received: 15/03/2023
Revised: 21/03/2023
Accepted: 25/03/2023
Published: 08/04/2023

Deepak Tomar, Dhananjay Taumar, Mayank, Prachi Jain
Assistant Professor, Kalka Institute for Research and Advanced Studies, Meerut (U.P), India 250005

Mainuddin, Anshika, Amulya Jindal
Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Meerut (U.P), India 250005

Khushi Goel
Assistant Professor, Meerut Institute of Technology, Meerut (U.P), India 250005

Correspondence

Amulya Jindal
Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Meerut (U.P), India 250005

Email: amulyajindal05@gmail.com

DOI: 10.24092/CRPS.2023.130105

Website: www.crpsonline.com

Quick Response Code:

Crocin as Neuroprotective Agonist in Oxidative Damage and Cognitive Dysfunction Induced by Aluminium Chloride and Colchicine in Rodents

Deepak Tomar, Mainuddin, Dhananjay Taumar, Anshika, Mayank, Prachi Jain, Khushi Goel, Amulya Jindal

ABSTRACT

The main constituent of saffron, Crocus sativus L., is called Crocin (CR). Water-soluble carotenoids in CR possesses anti-inflammatory, analgesic, anti-edematous, as well as anti-oxidant properties, as well as to increase the number of microtubules in sheep brain microtubules and to have neuroprotective effects. For example, Alzheimer's disease (AD), where the harm of hippocampal as well as cortical neurons results in memory as well as cognitive impairment, as well as amyotrophic lateral sclerosis (ALS), where weakness in muscle is brought on by the degeneration of spinal, bulbar, as well as cortical motor neurons, are disorders which are characterized by the progressive and irreversible loss of neurons. In the current investigation, we evaluated CR's ability to protect neurons from the oxidative harm that Colchicine (Col) and Aluminum chloride (ALCL) induce. GSH level, SOD, besides catalase reduced in the Col model's Col-treated group but was practically returned to normal in the group that received CR. When given CR instead of Col, the levels of MDA, acetylcholine esterase, and nitric oxide returned to nearly normal levels. However, in the Morris water maze test, CR significantly reduces the time it takes to reach the platform (escape latency time), indicating learning and cognitive improvement. Corresponding to this, in the passive avoidance test, the transfer delay time did not significantly increase in the CR-treated group but it did in the Col-treated group. Similar outcomes in the aluminium chloride model were attained. Thus, the present study comes to the conclusion that CR can be utilized to treat oxidative stress and illnesses linked to cognitive dysfunction, such as AD as well as Parkinson's diseases shown against rodents resultantly.

Key words: Crocin, Aluminium Chloride**,** Alzheimer's disease, oxidative stress, Neurodegeneration

1. INTRODUCTION

Alzheimer's disease (AD), which include the loss of hippocampal with cortical neurons that results in memory as well as cognitive impairment, Parkinson's disease (PD) and Huntington's disease (HD), which characterize the loss of neurons from basal ganglia structures results in abnormalities in the control of movement, and amyotrophic lateral sclerosis (ALS), in which the degeneration of neurons results in muscular weakness.

These diseases are fairly common and provide a significant medical and societal problem.[1,2,3] Colchicine (Col) is a potent anti-inflammatory, tumor growth inhibitor, and mitotic toxin. Col's ability to block microtubule polymerization by forming a complex with tubulin is likely the cause of the majority of its biological effects. Due to its severe toxicity, it is only occasionally employed in cancer treatment.[4,5,6] Attaching to tubulin, the main structural protein of microtubules, it causes neurofibrillary degeneration, which impairs learning and memory by leading to the loss of cholinergic neurons as well as decrease in acetylcholine transferase.[7,8] One of the chronic neurovirulent substances, aluminium, has an inhibitory effect on numerous brain processes, particularly learning and memory.[9] Aluminum chloride (ALCL) can alter the N-methyl-D-aspartate receptor's (NMDA) functions as well as the pellicular functions of nerve cells, which can disrupt cellular structure and function and, to varying degrees, impair learning and memory. One of the central glutamate's excitatory receptors, the NMDA receptor, is essential for the development of the cerebrum, learning, and memory processes, as well as synaptic plasticity and long-term improvement of the cortex and hippocampus.[10,11]

Sub chronic exposure to aluminium may impair learning as well as memory in developing rats, decrease the expression of NMDARG as well as PLC, and lesser the concentration of calcium, suggesting that the Ca signaling pathway may be disrupted as one of the mechanisms by which aluminium may impair learning and memory.[1,29] Aluminum modifies the function of NMDA receptors 1A and 2A/B on rat newborn hippocampus neurons. Hippocampal neurons were treated with 37, 74 M of Al for 14 days, which boosts the neural cell viability but inhibit the expression of NMDAR LA and NMDAR 2A/B. Hence it was proposed that exposure to Al may affect how hippocampus neurons mature.[10,13,14]

The primary as well as main active component of Crocus sativus L. is Crocin (CR) (Saffron) which is 3-5 cm in diameter, the saffron plant, Crocus sativus Linnaeus var. autamnalis, belongs to the Iridaceae family and has no stems (1,2). CR has been shown to have neuroprotective benefits in sheep brain microtubules, as well as tubulin polymerization and microtubule-increasing effects.[17,18,19] With regard to the learning and memory damage brought on by ethanol, CR has a unique, preventative impact.[20,21,22] The beginning of the NMDA type of glutamate receptors is primarily necessary for the formation of long-term potentiation (LTP) in the CAI and dentate gyrus areas of the hippocampus.[23,24,25] By opposing the inhibitory impact of ethanol on the NMDA receptor, CR is expected to counteract the suppression of hippocampus LTP caused by ethanol. CR shows effects, including anti-inflammatory, analgesic, anti-edematous, and anti-oxidant ones. It has also been suggested that CR has a role in the learning as well as memory processes.[15,26,27]

It has been documented that CR affects functional recovery following peripheral nerve damage. CR, a component of saffron, shielded the brain from severe oxidative stress in ischemia/reperfusion injury caused by global cerebral ischemia.[28,29,17] According to studies, CR and crocetin prevented the death of hippocampus cells caused by lipopolysaccharides (LPS) by lowering intracellular reactive oxygen species.[30,31,32]

The idea of neuroprotection is the injection of a substance that should undo or stop future neuronal damage brought on by the excitotoxicity of neurons. Certain substances offer defense against neuronal cell senescence.[33] Some substances, however, specifically defend the retina and dopamine neurons. Antioxidants make up the majority of neuroprotective substances. Moreover, two glycoprotein IIb/Illa antagonists and an estrogen agonist showed neuroprotective properties. The majority of synthesized chemicals were not initially intended to be neuroprotective agents, but later research revealed that they had neuroprotective effect.[34]

2. MATERIALS AND METHODS

2.1 Selection of Animal Species

Swiss albino mice of whichever sex (n=6) and young, healthy Wistar rats (n=6) were utilised as common laboratory strains. The female was not pregnant and was nulliparous. The animals' weight variations did not exceed 20% of the average weight for either sex. The typical weight of the rat was noted. (ALCL-induced neurotoxicity was studied using rats (150-200 g) whereas Col-induced neurotoxicity was studied using mice (25-35 g).

2.2 Accommodation and Husbandry

The animal room's temperature was held steady at 22°C (±3°C). A 50–60% relative humidity was maintained. There was no natural lightning; instead, there was a 12-hour cycle of light and darkness. For feeding, standard laboratory meals were employed, and water was available at all times for drinking. Animals were housed in small groups of the same sex and no more than six were kept in each cage while group caging.

2.3 Neuroprotective Action of Crocin Against Oral Dosage of Aluminium Chloride in Rats

2.3.1 Chemicals

Crocin (CR), aluminium chloride (ALCL), isotonic saline, Griess Reagent, bovine serum albumin, 0.8% thiobarbituric acid, 200 mM EDTA, sodium carbonate, hydroxylamine, heparinized saline, 4% paraformaldehyde, paraffin, and hematoxylin and eosin. CR dosage was chosen based on prior literature.[2]

2.3.1.1 Experimental Design

- ALCL and test drug formulations were freshly prepared before each experiment.
- ALCL and CR were dissolved in sterile water as well as normal saline, respectively, at a dose of 0.5 ml/100 g body weight for oral administration
- Each group consists of six animals, randomly assigned into five groups.

Group 1: Naive (received vehicle for aluminium chloride and CR)
Group 2: ALCL (100 mg/kg) + vehicle for CR
Group 3: CR (30mg/kg) + vehicle for ALCL
Group 4: CR (15mg/kg) + aluminium chloride (100 mg/kg)
Group 5: CR (30mg/kg) + aluminium chloride (100 mg/kg)

The investigation lasted 42 days (6 weeks). Then, by utilizing the locomotor activity, Morris water maze test, the passive avoidance paradigm, as well as biochemical estimates, we evaluated numerous behavioral parameters.[35,36]

2.4 Neuroprotective Action of CR Against Col Induced Neurotoxicity in Mice

Col is known to disrupt cognitive function and harm the brain's oxidative defenses in mice when administered intracerebrally.

2.4.1 Chemicals

Col, CR, Artificial cerebrospinal fluid (ACSF) (ACSF: 147 mM NaCl, 2.9 mM Kcl, 1.6 mM $MgCl_2$, 1.7 mM $CaC1_2$ and 2.2 mM dextrose).

2.4.2 Experimental Design

Seven groups consist of six animals per group were formed by randomly assigning the animals to those groups. Mice were placed in stereotaxic equipment and given thiopental sodium anesthesia (45 mg/kg ip) prior to operation. A sagittal incision was made on the scalp and two holes were drilled through the skull to insert the injection cannula into the lateral cerebral ventricles.

Group 1: Control mice treated with vehicle of CR.
Group 2: ACSF (10 µl i.c) + vehicle for CR
Group 3: Col treated group (3 ug/10 µl i.c) + vehicle for CR
Group 4: CR (15mg/kg) + ACSF (10 µl i.c)
Group 5: CR (30mg/kg) + ACSF (10 µl i.c)
Group 6: CR (15mg/kg) + Col (3 µg/10 ul i.c)
Group 7: CR (30mg/kg) +Col (3 ug/10 µl i.c)

On days 17, 18, 19, 20, and 21, behavioral tests such as the Morris Water Maze Test as well as all the Step Through Passive Avoidance Paradigm were then conducted, and locomotor activity was conducted every seventh day. On day 21 following the Col injection, oxidative stress markers were calculated by the help of final behavioral assessment.

2.5 Behavioral Assessment

2.5.1 Step Through Passive Avoidance Paradigm

It is a learning box with two compartments: a white compartment dimensions of 20 cm x 20 cm x 30 cm and a dark chamber (black compartment, 20 cmX20 cm X30 cm). The floor features a 6 cm × 6 cm opening for a guillotine door in the middle of the barrier between the two sections. Foot shock is produced by placing stainless steel grids (5 mm in diameter) on the floor of the dark room at Iem intervals (distance between grid centers).[37]

2.5.2 Evaluation of Spatial Memory by Morris Water Maze

A big, round, black pool of dimensions 120 cm in diameter and having a height of 50 cm, which is full to a depth of 30 cm by water that is 26+2 °C makes up the Morris water maze. A spherical platform of black color, having a diameter of 8 cm, was fixed in place 1 cm below the surface of water. To conceal the position of submerged platform, tint the water a non-toxic shade of black.[32,38,39] We should split the pool into four fictitious quadrants. A five-day trial should be conducted with Morris Water Maze to train mice. A rat is allowed to locate the hidden platform for 60 seconds (the cut-off time) and to remain on it for 30 seconds. If the rat is unable to find the platform, the experimenter will place the rat there by himself. Each trial's latency time to reach the platform was noted. The findings display the mean delay time for each of the three trials. A successful learning outcome was defined as a significant reduction in latency time from that of the first session.[40,41,42]

2.5.3 Locomotor Activity

An actophotometer was used to record the locomotion. Animals were individually positioned in the activity meter for 2 minutes before the locomotor challenge to let them become used to it. Following that, an actophotometer was used to record locomotor activity for five minutes.[37,43]

2.6 Estimation of Biochemical Parameters

The final behavioral task was followed by biochemical tests. Decapitation was used to sacrifice the animals. After being rinsed with ice-cold isotonic saline, the brain was removed and homogenized using 0.1 mmol/l phosphate buffer (pH 7.4). The homogenate (10% weight to volume) was centrifuged at 10,000 rpm for 15 minutes and used as the biochemical sample.[18,19]

2.6.1 Estimation of Acetylcholinesterase (AChE) Activity

We utilised Wistar rats of either sex, weighing about 160 g. To prevent any tissue damage, they were carefully put to death by cervical dislocation. Using the Ellman approach, the AChE activity over the entire brain was assessed (12). DTNB (5,3-dithiol-bis (2-nitrobenzoic acid) (Ellman' reagent) (0.01%), acetylthiocholine iodide, 3 ml of phosphate buffer, and 0.1 ml of homogenate were all included in the assay mixture. Every minute for two minutes starting at 412 nm, the absorbance was measured. Micromoles of acetylcholine iodide hydrolyzed/minute/mg protein were used to express the AChE activity.[45,46]

2.6.2 Estimation of Nitrite

Five percent phosphoric acid, 1 percent sulfanilamide, and N-(1-naphthyl) ethylenediamine dihydrochloride were employed in a colorimetric experiment to quantify the concentration of nitrite in the supernatant, a marker of nitric oxide production.[47,48] At room temperature and in the dark, equal parts of supernatant and Griess reagent were combined and incubated. A spectrophotometer was used to measure the absorbance at 540 nm. The quantity of nitrite in the supernatant was calculated using a sodium nitrite standard curve.[49,50]

2.6.3 Protein Estimation

By using bovine serum albumin as a reference, the protein content of the brain homogenate was assessed using Lowry's technique. The protein concentration was indicated as mg/ml of homogenate.[51,52,53]

2.6.4 Estimation of Lipid Peroxidation

By using the Ohkawa et al approach, MDA was carried out. Lipid peroxidation produces MDA as a byproduct, which interacts with thiobarbituric acid to produce a light pink result. Supernatant was measured out to be ml.[54] Test tubes were filled with 0.3ml of 0.8 percent thiobarbituric acid (TBA) and 0.5ml of 30 percent trichloro acetic acid (TCA). Tubes were covered in aluminium foil and submerged in the water bath for 30 minutes at 80°C. The combination was then submerged for 30 minutes in ice-cold water. After cooling, the color was extracted in ml of butanol, and the UV spectrophotometer was used to detect the intensity at 540 nm.[55,56,57]

2.6.5 Estimation of GSH

1ml of supernatant and Iml of 10% TCA were used in the test. This mixture was centrifuged at 3000–4000 gyrations for 10 minutes. A second collection of the supernatant was made, and 10ul of it was mixed with 2ml of 0.1M phosphate buffer (pH-7.4), 0.5ml of DTNB (5,5'-dithiol-bis (2-nitrobenzoic acid), and 0.4ml of distilled water. The absorbance of this mixture was measured at 412 nm after 15 minutes.[44,58]

2.6.6 Estimation of SOD

The assay system included four solutions: solution-A, which contained 50 mM sodium carbonate in 0.1 mM EDTA (pH-10), solution-B, which contained 96 uM nitro blue tetrazolium in solution-A, solution-C, which contained 0.6% Criton X-100 in solution A, and solution-D, which contained 20 mM hydroxylamine.[59,60,61] Standard was composed of 1.3ml of Sol-A, 1.1ml of Distilled Water, 0.5ml of Sol-B, 0.1ml of Sol-C, and 0.1ml of Sol-D. In the test, 1.3 ml of solution A, 1 ml of distilled water, 0.5 ml of solution B, 0.1 ml of solution C, and 0.1 ml of solution D were ingested. 1.3 ml of sol A, 1 ml of distilled water, 0.5 ml of sol B, 0.1 ml of sol C, and 0.1 ml of supernatant were obtained from the blank sample. At 560nm, the absorbance was measured.[11,62,63]

2.6.7 Estimation of Catalase

The Luck method, which measures the rate at which hydrogen peroxide breaks down, was used to gauge catalase activity.[51,64,65] The supernatant of the tissue homogenate and 3 ml of H2O2 phosphate buffer were quickly combined. For two minutes, the absorbance change at 240 nm was measured every 60 seconds. The results were expressed as micromoles of hydrogen peroxide decomposed/min/mg of protein.[66,67]

2.6.8 Histological examination

Following the behavioural test, rats were sacrificed by deep anaesthesia with pentobarbital (50 mg/kg, ip), transcardial perfusion with cold heparinized saline, and 4% paraformaldehyde. The brain was then taken out of the skull, post-fixed in paraformaldehyde for an overnight period, then dried out and embedded in paraffin. Using a rotary microtome, representative coronal slices (5-pm-thick), containing the dorsal hippocampus, were cut out and stained with hematoxylin and eosin (HE). In order to identify neuronal injury in the hippocampus, microscopic analysis of the CAI's neuronal density was performed.[43,68]

2.7 Statistical Analysis

Analysis of variance (ANOVA) and the Dunnetts test were used in the statistical analysis of the data from the aforementioned study.[69,70]

3. RESULTS

3.1 CR's Neuroprotective Action of Col Induced Neurotoxicity in Mice Against Intracerebral Administration.

3.1.1 CR's Effect in Morris water Maze Test on Col Induced Memory Impairment

In the Morris water maze, it was noticed that the animals in the Normal control, ACSF-injected, and CR per se (15 and 30 mg/kg, ip) groups quickly learnt to swim directly to the platform. The escape latency time was longer in mice that had received Col injections. The escape latency time significantly decreased by CR (15 and 30 mg/kg ip).

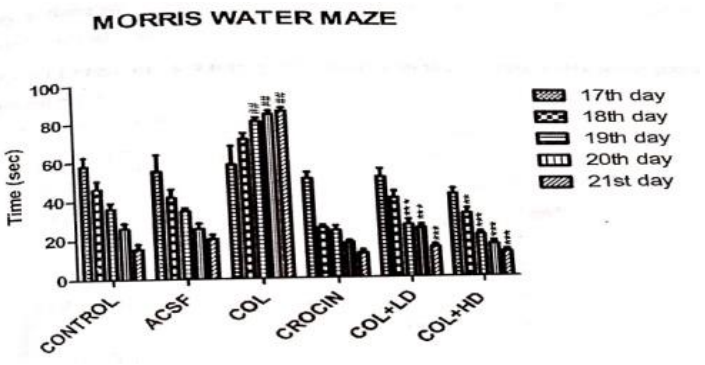

Figure 1.1: CR effect on Morris Water Maze Test

Figure 1.1 depicts normal control and ACSF-treated mice, both of which demonstrated a significant decrease in escape latency time (ELT) starting in session three, showing spatial learning. Col was injected intracerebrally at a dose of 3µg/mice to cause memory loss. On day 17 to day 21 after Col administration, memory function was assessed. Col demonstrated a substantial increase in ELT at sessions 3, 4, and 5 when administered at a dose of 3 µg [F (4, 25) = 5.969, P <0.001].[71,72]

Four days before the Col injection, CR doses of 15 and 30 mg/kg were administered for 25 days. From session 3 onward, mice given CR 15 mg/kg displayed a substantial drop in ELT (F (4, 25) = 22.05, P <0.001). From session 3 onward, greater doses [F (4, 25) = 40.21, P <0.001] also lessen ELT. Moreover, the CR per se therapy enhanced spatial memory as seen by a significant decrease in ELT beginning in session 3 compared to session 1 [F (4, 25) = 52.31, P <0.001]. (Displayed in Table 1.1).

Legend: CR and Col's effects on mice's spatial memory (n = 6). Five sessions of three trials each in a water maze were administered to mice. The findings were shown as mean latency time (s) + S.E.M. (n=6). One-way ANOVA was used to evaluate the data, and Dunnet's test was used to make comparisons with session I. Substantial difference from session 1 in latency time (***p <0.001, **P <0.01 and *P <0.05).

3.1.2 CR's Effect in Passive Avoidance Test on Col Induced Memory Impairment

The 1 considerably increased the transfer latency time "and two retention trials in comparison to the acquisition trial in the control group (F (4, 25) = 15.85, P <0.001) and ACSF group (F (4, 25) = 15.25, P <0.001) groups. No discernible increase in TLT in the 1-hour TLT was seen in the Col (3 g) treated group "the acquisition trial [F (4, 25) 0.6351, P > 0.001] and second retention trials. A lower dose of CR (15 mg/kg) had a low significant impact on improving TLT in retention trials [F (4,25) = 17.76, P <0.001], although the TLT of retention trials was significantly greater in the CR (30 mg/kg) treated group than that of the acquisition trial. Figure 1.2 Table 1.2, which summarizes the trials, is provided below.

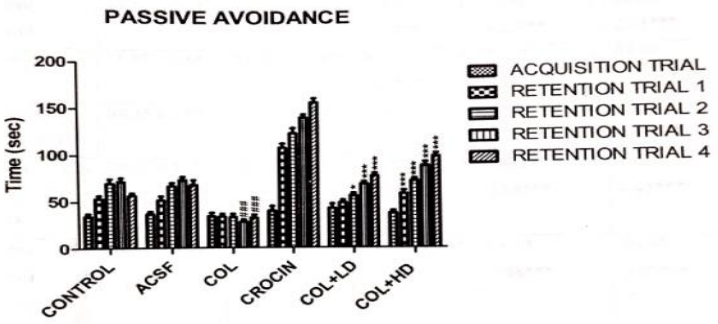

Figure 1.2: CR effect on Passive avoidance paradigm

Legend: Effects of Col and CR on a mouse activity involving passive avoidance. The mean transfer latency time (s) plus SEM was used to express the results. (n=6). One-way ANOVA was used to examine the data, and Dunnet's test was used to compare results to acquisition trials. a substantial difference in transfer latency time compared to the acquisition trial (***p <0.001, **P <0.01, *P <0.05).

3.1.3 CR's Effect on Locomotor Activity in Col (i.c) Induced Memory Deficits

The mean scores of each animal's locomotor activity in the current set of studies were essentially constant and did not significantly differ across the various groups. Normal control, ACSF, and Col-treated mice all continue to have the same mean scores. Moreover, neither of the CR doses (15 or 30 mg/kg i.p.) significantly changed the mice's locomotor activity on days 14 and 21 compared to those who received Col (Table 1.3 and Figure 1.3).

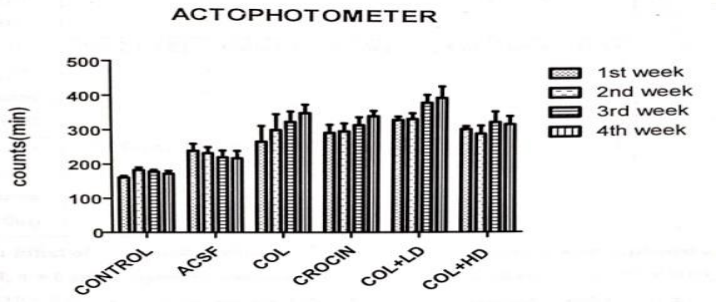

Figure 1.3: CR effect on locomotor activity of mice

Legend: Col and CR's impact on locomotor activity. Data were presented as mean SEM, n-6, and one-way ANOVA analysis was used before Dunnet's test. When High dose and Low dosage groups are compared with Col 3 g, *P<0.05, **P <0.01, and ***P <0.001 are obtained.

3.1.4 Estimation of Biochemical Parameters

Data were shown as mean S.E.M. n-6, and one-way ANOVA was used to examine the data before Dunnett's test. On comparing both High dose and Low dose groups to Col 3 µg, *P <0.05, **P <0.01 and ***P <0.001 are obtained. When group Col 34 g is compared to Normal control and ACSF groups, #P <0.05, ##P <0.01 and ###P <0.001 are obtained.

3.1.4.1 CR's Effect on Malondialdehyde (MDA) Level

On day 21 after starting Col, the brain's MDA concentration (nmol/mg protein) was assessed. As comparison to the normal control group of mice (0.81±0.02 nmol/mg protein) and the ACSF group (0.98 ± 0.07 nmol/mg protein), mice treated with Col (3µg) had considerably higher levels of MDA (5.96±0.59 nmol/mg protein, P <0.001). When compared to the Col group (5.96 ± 0.59 nmol/mg protein), the levels of MDA were significantly lower in the groups treated with 15 mg/kg and 30 mg/kg CR (2.50±0.22 nmol/mg protein) respectively (Figure1.4: Table 1.4).

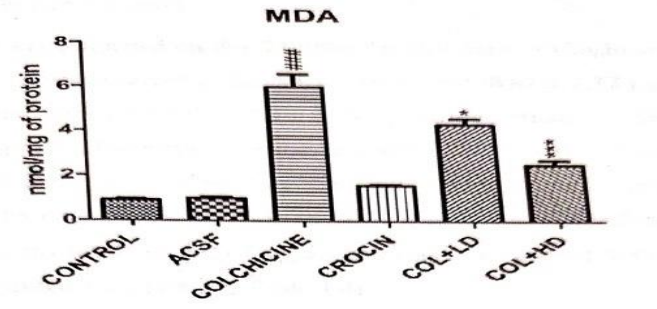

Figure 1.4: CR effect in mice brain on MDA (nmol/mg protein) level.

3.1.4.2 Effect of CR on Nitrite Level

On day 21 after starting Col, the brain's nitrite concentration (µg/mg protein) was assessed. When compared to the untreated control group (16.40±0.86 ug/mg protein) and the ACSF group (14.57±1.61 pg/mg protein), the amount of nitrite considerably increased in the mice treated with Col (3 µg) (36.104.17 ug/mg protein, P <0.001). When compared to animals given Col (36.10±4.17 µg/mg protein), the nitrite levels in the 15 mg/kg CR-treated group reduced, but not significantly, and in the 30 mg/kg CR-treated groups (21.95±0.98 µg/mg protein, P <0.01) (Figure 1.5 and Table 1.4).

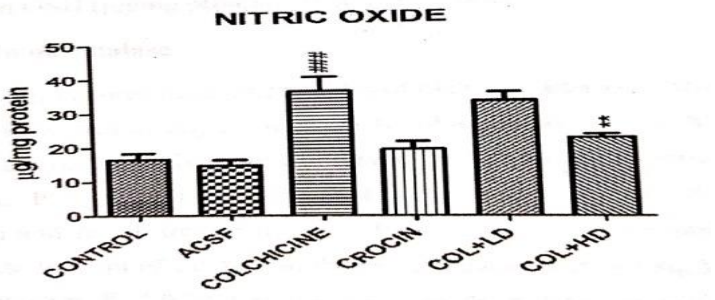

Figure 1.5: CR effect on nitrite (µg/mg protein) level in mice brain.

3.1.4.3 CR effect on GSH Level

On day 21 following the 3µg Col dose, the amount of GSH (ng/mg protein) was calculated. When compared to the groups who received ACSF treatment (45.82±5.80 µg/mg protein) and normal controls (44.90 ± 5.40 µg/mg protein), GSH levels in the Col group (20.67±2.37 µg/mg protein, P <0.05) significantly decreased. In contrast to the Col group (20.67±2:37 µg/mg protein), there was an increase in GSH levels in the group treated with 15 mg/kg CR (25,79 ±2.62 µg/mg protein). On the other hand, GSH levels significantly increased in the 30 mg/kg CR (35.92±2.67 µg/mg protein) treated group with as compared to the Col group (20.67± 1.38 µg/mg protein) (Table 1.4 in Figure 1.6).

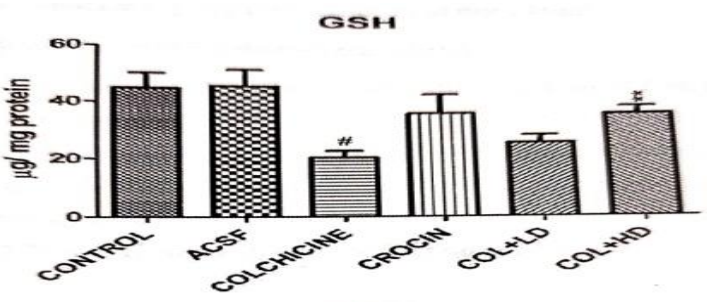

Figure 1.6: CR effect on GSH (µg/mg protein) level in mice brain.

3.1.4.4 CR's Effect on Catalase

µM of H_2O_2degraded per minute per mg of protein were used to express catalase's results. On the 21st day following the Col 3µg dose, an estimate was made. In contrast to the normal control (0.419±0.039) and ACSF treated (0392±0.023) groups, a substantial decrease in the levels of catalase enzymatic activity was seen in the Col group (0.093±0.016 mol/min/mg protein, P<0.001). When compared to the Col group (0.093±0.016 µmol/min/mg protein), there was a significantly higher level of catalase in the group treated with 30 mg/kg CR (0.206±0.015 mol/min/mg protein, P<0.001). 4.4 (Figure1.7, Table 1.4).

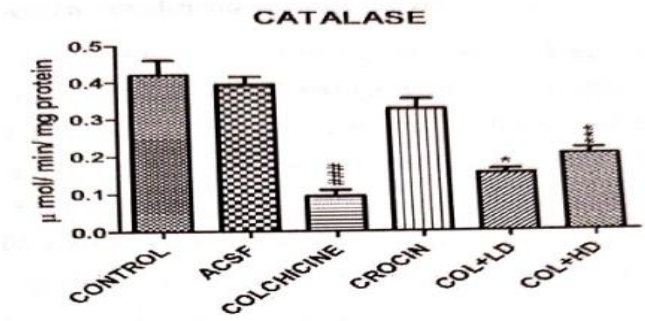

Figure 1.7: CR effect on Catalase (ug/mg protein) level in mice brain.

3.1.4.5 CR Effect on Superoxide Dismutase (SOD)

On day 21 following the 3ug Col dose, SOD (units/mg protein) levels were estimated. In comparison to the untreated ACSF group (80.77±16.24 units/mg protein) and the normal control group (81.14±20.68 units/mg protein), a substantial downfall in SOD levels observed in the Col group (12.76±4.17 units/mg protein, P <0.05). On comparison to the Col group (12.76±4.17 units/mg protein), there was a significantly higher level of SOD in the group treated with 30 mg/kg CR (64.75±14.97 units/ mg protein, P<0.01) (Figure1.8: Table 1.4).

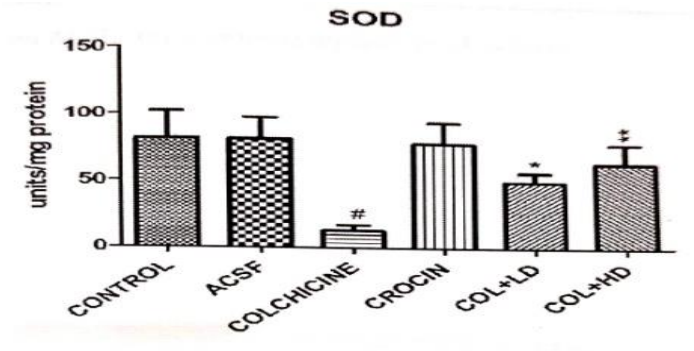

Figure 1.8: CR effect on SOD (units/mg protein) level in brain.

3.1.4.6 Effect of CR on Acetylcholine Esterase (ACHE)

On day 21 following the 3 µg Col treatment, the ACHE (mol/min/mg protein) was calculated. When compared to the groups receiving ACSF treatment (0.00031±0.000005 mol/min/mg protein) and the untreated control (0.000318 ± 0.000003 mol/min/mg protein), the levels of ACHE were significantly more in the Col (0.0090 ± 0.00066 mol/min/mg protein, P<0.001) group. When compared to the Col (0.0090±0.00066 mol/min/mg protein) group, the amount of ACHE was significantly lower in the groups treated with both 15 mg/kg and 30 mg/kg of CR (0.00606±0.00044 mol/min/mg protein, P <0.001) and 0.00321 ±0.00013 mol/min/mg protein, P <0.001. (as shown in Figure 1.9, Table 1.4).

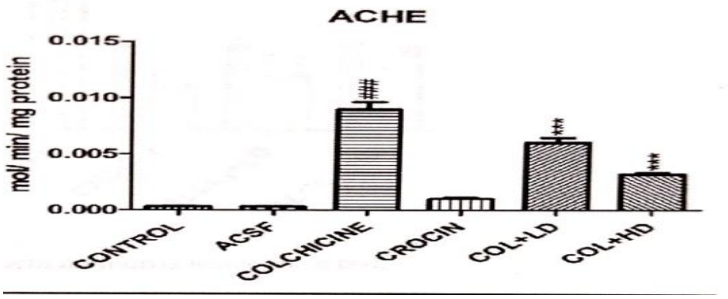

Figure 1.9: CR effect on ACHE (mol/min/mg protein) level in brain

3.2 CR's Neuroprotective Properties Against the Neurotoxicity that Oral Aluminium Chloride Treatment Causes in Rats

3.2.1 CR Effect on Aluminum Chloride-Induced Memory Impairment in Morris Water Maze Test

The normal control-treated rats were shown to exhibit a substantial reduction in escape latency time (ELT) from session two onward, showing spatial learning [F (2,15) = 6.09, P <0.05]. ALCL was ingested by rats at a level of 100 mg/kg to cause memory loss. The ability to recall information was tested on days 20, 21, and 42. At sessions 2 and 3, a dosage of aluminium chloride 100 mg/kg [F (2, 15) =6.54, P<0.01] significantly increased ELT.

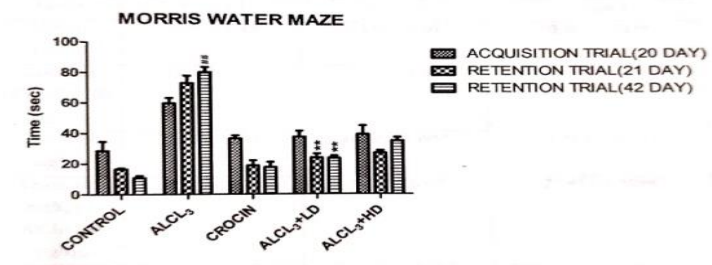

Figure 1.10: CR effect on morris water maze test.

From the day of aluminium chloride administration for 42 days, CR was given in doses of 15 and 30 mg/kg. From session 2 on, the ELT of the CR 15 mg/kg (F (2,15) = 7.66, P < 0.01] treated rat significantly decreased. At a higher dose of 30 mg/kg (F (2. 15)= 2.86), the ELT also decreased from session 2 on, but not significantly. Moreover, CR per treatment improved spatial memory as seen by a significant drop in ELT from session 2 onward compared to session 1 [F (2, 15) = 12.64, P<0.001]. (as shown in Figure 1.10 and Table 1.5).

Legend: Impact of CR and ALCL on rats' spatial memory (n = 6). On days 20, 21, and 42, rats underwent three sessions of three trials each in a water maze. Mean latency time (s) and S.E.M. (n=6) were used to express the results. One-way ANOVA was used to evaluate the data, and Dunnett's test was used to compare results to session 1. A notable variation in latency time from session 1 (***p <0.001, P <0.01, *P <0.05).

3.2.2 CR Effect on ALCL, Induced Memory Impairment in Passive Avoidance Test

The 1 considerably increased the transfer delay time "compared to the acquisition trial in the control, and 2 retention trials (F (2, 15) =57.85, P<0.001). There was a substantial drop in TLT in the ALCL, (100 mg/kg) treatment group, with the 1 "in addition to two retention trials [F (2, 15) =5.98, P <0.05] as compared to the acquisition trial. While a lower dose of CR (15 mg/kg) had a less significant effect on improving TLT in retention trials (F (2, 15) =5.80, P<0.05), the TLT of retention trials was significantly greater in the CR (30 mg/kg) treated group than it was in the acquisition trial. (Figure 1.11 and Table 1.6), which summarizes the trials, is provided below.

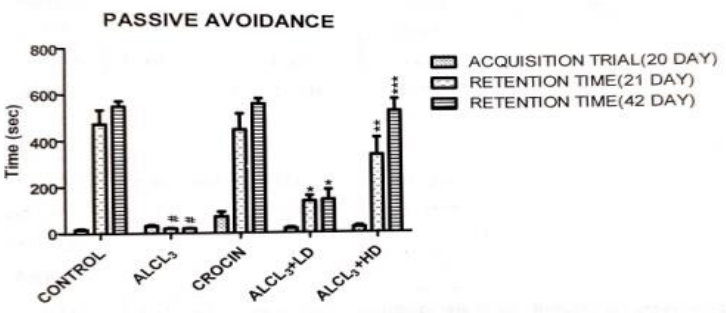

Figure 1.11: CR effect on Passive avoidance paradigm.

Legend: Effects of CR and AlCl3 on rats doing a passive avoidance exercise. Mean transfer delay time (s) S.E.M. was used to express the results (n 6). One-way ANOVA was used to examine the data, and Dunnet's test was used to compare results to acquisition trials. a substantial variation in transfer delay time compared to the acquisition trial (***p <0.001, P <0.01, *P <0.05).

3.2.3 CR Effect on Locomotor Activity in ALCL, Induced Memory Deficits

The mean scores of each animal's locomotor activity in the current set of studies were essentially constant and did not significantly differ across the various groups. The average scores in the ALCL-treated and normal control rats are unchanged. Additionally, neither of the CR doses (15 and 30 mg/kg, i.p) significantly changed the mice's locomotor activity on days 35 and 42 compared to mice treated with ALCL (Table 1.7 and Figure 1.12).

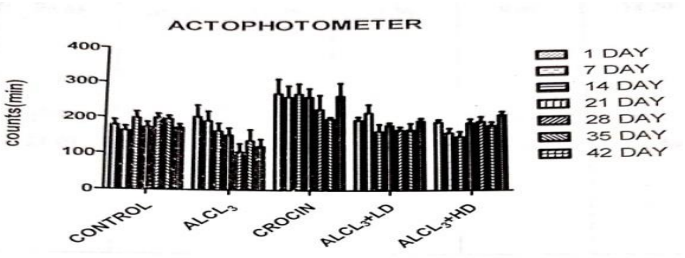

Figure 1.12: CR effect on locomotor activity.

Legend: CR and ALCL's impact on the ability to move. Data were presented as mean SEM. n-6, and one-way ANOVA was used to examine them before Dunnett's test. When High dose and Low dosage groups are compared with ALCL, 100 mg/k, *P <0.05, **P <0.01, and ***p <0.001 are obtained.

3.2.4 Biochemical Parameters Estimation

3.2.4.1 CR Effect on Malondialdehyde (MDA) Level

On day 42 of ALCL, the MDA concentration (nmol/mg protein) in the brain was determined. Rats treated with ALCL (100 mg/kg) had significantly higher levels of MDA (1.55±0.165 nmol/mg protein vs. 0.420±0.026 nmol/mg protein, P <0.001). The MDA level was significantly reduced with 30 mg/kg CR compared to ALCL-treated rats (1.55 ±0.165 nmol/mg protein) but less dramatically with 15 mg/kg CR than with 30 mg/kg CR (0.79±0.03 nmol/mg protein, P<0.001). (Table 1.8 in Figure 1.13).

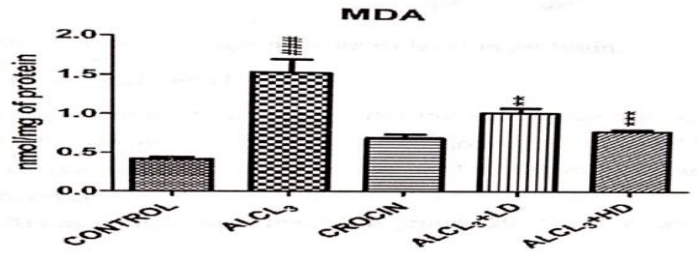

Figure 1.13: CR effect on MDA (nmol/mg protein) level in rat brain.

3.2.4.2 Effect of CR on Nitrite Level

The Nitrite level (µg/mg protein) in the brain was measured on day 42 of ALCL. The level of nitrite increased significantly in ALCL, (100 mg/kg) treated rats (1.55±0.16 µg/mg protein, P < 0.001) as compared to normal control (0.42± 0.03 µg/mg protein) treated. On the other hand, both 15 mg/kg CR (1.04 ± 0.06 µg/mg protein, P<0.01) and 30 mg/kg CR (0.80 ± 0.03 µg/mg protein, P<0.001) treated groups significantly decreased nitrite level as compared to ALCL, treated rats (1.55±0.16 µg/mg protein). (Figure: 1.14 Table 1.8).

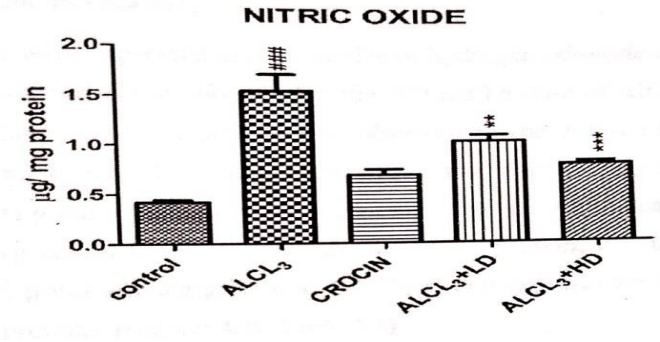

Figure 1.14: CR effect on nitrite (pg/mg protein) level in rat brain.

3.1.4.3 Effect of CR on GSH Level

GSH (µg/mg protein) was estimated on day 42 after the 100 mg/kg dose of ALCL, A significant fall in the levels of GSH was observed in the ALCL, group (20.99±3.42 pg/mg protein, P< 0.001) as compared to the normal control (53.30±4.47 ug/mg protein) group. There was a significant increase in the level of GSH in the group treated with 30 mg/kg CR (34.99 ± 3.01 ug/mg protein, P<0.01) as compared to the ALCL, group (20.99 ± 3.42 µg/mg protein). (Figure 1.15, Table 1.8).

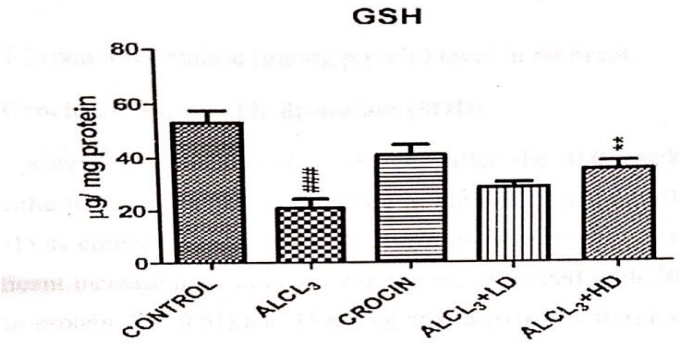

Figure 1.15: CR effect on GSH (ug/mg protein) level in rat brain.

3.2.4.4 Effect of CR on Catalase

Micromoles of hydrogen peroxide degraded per minute per mg of protein were used to express catalase's results. On day 42 following the administration of ALCL at a dose of 100 mg/kg, when compared to the normal control group (0.200±0.006 mol/min/ing protein), the levels of catalase enzyme activity were significantly lower in the ALCL group (0.036±0.004 mol/min/mg protein, P <0.001). When compared to the ALCL group (0.036±0.004 mol/min/mg protein), the levels of catalase significantly increased in both the 30 mg/kg and 15 mg/kg CR-treated groups (0,130±0.020 mol/min/mg protein, P<0.001; 0.84±0.005 mol/min/mg protein, P<0.05). (Table 1.8, Figure 1.16).

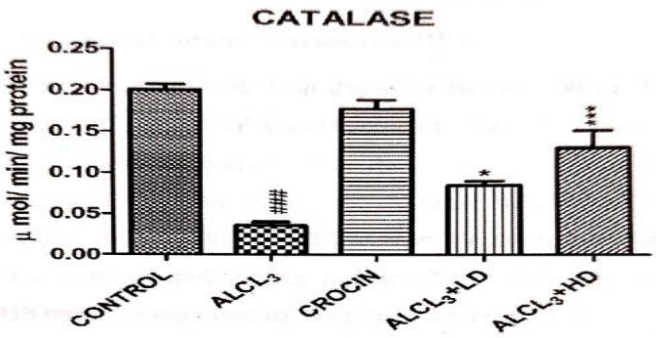

Figure 1.16: CR effect on Catalase (pg/mg protein) level in rat brain.

3.2.4.5 Effect of CR on Superoxide Dismutase (SOD)

On day 42 following the 100 mg/kg dose of ALCL, SOD (units/mg protein) was measured. Compared to the healthy control group (0.200±0.006 units/mg protein), the levels of SOD in the ALCL group significantly decreased (0.036±0.025 units/mg protein, P <0.001). In comparison to the ALCL group (0.036 ±0,004 units/mg protein), there was a significantly higher level of SOD in the groups treated with 30 mg/kg and 15 mg/kg CR (0.179±0.010 units/mg protein, P<0.01, and 0.099±0.011 units/mg protein, P<0.05). (Table 1.8, Figure 1.17).

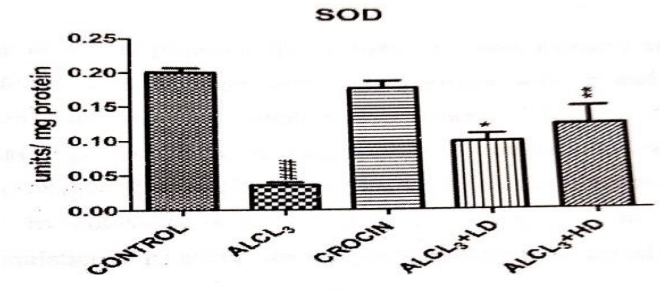

Figure 1.17: CR effect on SOD (units/mg protein) level in brain.

3.2.4.6 Effect of CR on Acetylcholine esterase (ACHE)

On day 42 following the administration of 100 mg/kg of ALCL, ACHE (mol/min/mg protein) was measured. When we compare with the normal control group (0.00024±0.0000019 mol/min/mg protein), the levels of ACHE in the ALCL group were significantly higher (0.0076±0.00038 mol/min/mg protein, P<0.001). When compared to the ALCL group (0.0076± 0.00038 mol/min/mg protein), the amount of ACHE was significantly lower in the groups which treated with both 15 mg/kg and 30 mg/kg of CR (0.00520±0.00039 mol/min/mg protein, P <0.001) and 0.00103±0.00016 mol/min/mg protein, P <0.001. (Table 1.8, Figure 1.18).

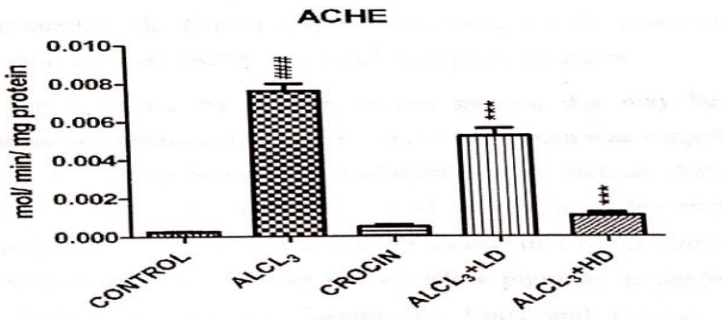

Figure 1.18: CR effect on ACHE levels in brain (mol/min/mg protein).

4. DISCUSSION

Attention, memory, learning capacity, language production and comprehension, reasoning, problem-solving, and decision-making are all parts of the group of mental activities known as cognition. The pathogenesis of many neurological illnesses, particularly neurodegenerative ones, has been linked to oxidative stress (OS). Since OS oxidize essential cellular components like lipids, proteins, in addition DNA, it results in cellular damage and ultimately cell death. The neurotransmitter glutamate can cause excitotoxicity when it is exposed to excessive amounts or overstimulated at its membrane receptors.

Reactive oxygen/nitrogen species (ROS) or else their precursors are scavenged, metal ions are necessary for the catalysis of ROS formation, and natural antioxidant defenses are triggered. ROS generation is blocked. Exogenous antioxidants, on the other hand, may be quite efficient in reducing the cumulative consequences of oxidative damage given that our endogenous antioxidant defenses are not always fully effective and that we are being exposed to more harmful environmental variables. Although a variety of variables can cause OS in cells, glutamate, a neurotransmitter, is principally responsible for this process in the brain by activating its ionotropic receptors. The bulk of excitatory synaptic activity in the human brain is caused by glutamate and other excitatory amino acids, which are released by around 40% of all synapses. The NMDA, a-amino-3-hydroxy-5 methyl-4-isoxasoleproprionic acid (AMPA), and kainic acid (KA) receptors can be distinguished from other ionotropic receptors based on their pharmacological and electrophysiological properties.

Many medications have the ability to control ROS, which may be utilized to treat memory as well as cognitive problems. CR was recommended as a result due to its antioxidant qualities. CR has a wide spectrum of pharmacological properties, including anti-inflammatory, analgesic, as well as anti-edematous capabilities. It has also been suggested that CR has a role in the learning as well as memory processes. The essential ingredient in Crocus sativus L. is CR (Saffron). Water soluble carotenoid is CR. The yellow pigment CR may be found in saffron as well as gardenia yellow, which are extracts of the stigmas of Crocus sativus as well as Gardenia jasminoides, respectively. When tested on biomarkers and behavioral factors, CR exhibits substantial alterations.

The results of the present study confirmed that CR has a neuroprotective effect when administered intraperitoneally. Evaluation of numerous biochemical and behavioral characteristics led to the suggestion. Col as well as aluminium chloride-induced oxidative damage as well as cognitive dysfunction were used as two models in this investigation to assess the neuroprotective effect of the CR.

Actophotometer results in no appreciable decrease in locomotion in Swiss Albino mice treated with Col, demonstrating that the drug has no effect on locomotor activity. Contrarily, in the Morris water maze test, the escape latency time of the CR-treated group was much lower than that of the Col-treated group, representing that Col shows neurodegeneration whereas CR shows a protective effect against Col. Similar to the active avoidance paradigm, the Col-treated group had a substantial decrease in transfer latency time.

Analysis of biochemical parameters revealed that the Col-treated group had higher levels of MDA, Acetylcholine esterase, as well as Nitrite and lower levels of GSH, SOD, as well as Catalase, demonstrating that Col induces neurodegeneration. High doses of GSH (30 mg/kg, i.p) meaningly higher levels of GSH relative to the group receiving Col. When compared to the Col-treated group, the SOD level was considerably higher in both the low (15 mg/kg) and high (30 mg/kg) dosages of CR. Similarly, at both low dosages of CR (15 mg/kg) and high doses, catalase levels were significantly higher. Both the low (15 mg/kg) and high (30 mg/kg) doses of CR had considerably lower levels of MDA than the group receiving Col. Also, it was discovered that the AChE level was much lower than in the group that received Col. Moreover, the amount of nitrite was much lower than it was for the group receiving Col. According

to the outcomes of the current investigation, CR at a higher dose (30 mg/kg) was superior than a low dose (15 mg/kg) for neuroprotection. Additionally, identical behavioral and metabolic characteristics were assessed in an aluminium chloride-induced neurotoxicity model.

Similar to Col, no locomotor incoordination was seen in the ALCL-treated rats. When compared to ALCL, the treated group, the CR-treated group's escape latency time in the Morris water maze test was much lower. Similar to the active avoidance paradigm, the ALCL-treated group's transfer latency time has significantly decreased.

The identical outcomes as those of the Col-induced neurotoxicity model are revealed by biochemical measures. ALCL is thought to cause neurodegeneration since GSH, SOD, and catalase levels fall in ALCL-treated groups whereas MDA, AChE, and nitrite levels rise in AICh-treated groups. When compared to the AlCl-treated group, the High dosage treatment group (30 mg/kg) had significantly greater levels of GSH, SOD, as well as catalase. The level of MDA was observed to be considerably lower in the high-dose treated group compared to the AlCl treated group. When compared to ALCL, the control group, both the low (15 mg/kg) and high (30 mg/kg) dose-treated groups of CR had considerably reduced levels of AChE and nitrite. Consequently, it can be inferred from the ALCL-induced neurodegeneration model that CR at large dosages is more effective than at low doses at preventing neurodegeneration.

Table 1.1: CR effect on memory performance for Col treated mice in the Morris water maze paradigm

Escape Latency Time (sec)					
Group	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**SESSION 5**
Normal Control	57.09 ± 4.43	46.10 ±4.06	36.93±2.72***	26.71±2.65***	16.32±2.37***
ACSF	53.4± 7.56	41.21±4.05	34.21±1.54*	25.16±2.66***	20.0±2.10***
Col (3µg 10µl)	56.46±8.42	70.38 3.09	79.49±1.76***	83.10±1.87***	84.88±1.56**
CR (30 Mg/Kg) + ACSF	49.21 ±2.13	25.32 ± 1.32	23.82±2.31***	17.66±0.95***	12.38±1.51***
CR (15mg /Kg) +Col (3µg/10µl)	49.88±4.70	40.22 ±3.41	26.49±2.48***	24.93±1.75***	15.16±1.21***
CR (30 Mg/Kg) + Col (3µg/10µl)	43.27±2.59	31.26±3.42**	20.33±1.41***	17.43±1.58***	13.54±2.04***

Table 1.2: CR effect in Passive avoidance test on Col induced memory impairment

Group	**Acquisition trial**	**Retention trial 1**	**Retention trial 2**	**Retention trial 3**	**Retention trial 4**
Normal Control	33.31 ± 2.93	51.32 ±3.37**	68.82±4.65 * * *	70.5±4.21 ***	55.82±3.62 ***
ACSF	35.82 ± 2.58	50.49 ± 4.37*	65.66±3.52 * * *	72.35±3.42 * * *	67.00±5.25* * *
Col(3µg/10µl)	33.32 ± 3.62	32.82 ± 2.82	32.05 ± 3.61	27±2.55***	32.17±344* **
CR (30mg/kg) +ACSF	38.67 ± 5.19	106.5 ±4.58 * * *	1223.2±5.60* * *	141.2±3.54* **	155.7±4.43 * * *
CR (15mg/kg) Col(3µg/10µl)	42.17 ± 4.96	48.17±2.92	55.50 ± 2.96*	68.332.58* **	76.83±2.87 ***
CR (30mg/kg) Col(3µg/10µl)	36.83 ± 2.82	57.50 ±3.74 * * *	71.17±2.81 ***	87.33±4.10***	97.17±3.34* **

Table 1.3: Effect of Col and CR on Locomotor Activity

Group	**1st DAY**	**7th DAY**	**14th DAY**	**21th DAY**
Normal Control	160.1 ± 4.48	181.7 ± 7.07	177.0± 5.43	172.4 ± 8.71
ACSF	238.6 ± 20.16	236.6 ± 17.07	220.2± 19.43	217.2±21.37
Col (3pg/10µl)	265.6 ± 43.12	300.0 ± 45.03	323.2±30.19	350.2±24.71
CR (30 mg/kg) +ACSF	292.0 ± 22.29	296.3 ± 16.43	314.5 ± 22.02	340.4± 14.60
CR (15 mg/kg) +Col (3µg/10µl)	330.2±8.82	334.0 ± 15.61	380.3 ± 22.13	394.7±32.22
CR (30 mg/kg) + Col(3µg/10µl)	302.4 ± 7.82	289.1 ± 22.34	322.4±30.19	316.4±21.41

Table 1.4: CR effect on oxidant- antioxidant level in mice brain

Groups	**GSH**	**MDA**	**NITRIC OXIDE**	**SOD**	**ACHE**	**CATALASE**
Normal control	44.90 ±5.39	0.91 ± 0.04	16.40 ±1.86	81.14±20.68	0.000318 ±0.0000036	0.419 ±0.0399
ACSF	45.82 ±5.76	0.98 ± 0.07	14.57 ±1.61	80.77 ±16.24	0.000315 ±0.0000056	0.393 ±0.0226
Col(3pg/10yl)	20.67 ±2.37#	5.957 ±0.59###	36.10 ±4.17###	12.76±4.17#	0.00904 ±0.000663###	0.093 ±0.0157###
CR (30mg/kg) + ACSF	36.34 +6.72	1.56 + 0.06	19.01+ 2.24	78.82 +15.85	O. OOIOO ±0.000142	0.327 +0.0278
CR (15mg/kg+ Col (3µg/10µl)	25.79 ±2.62	4.29±O. 26*	32.78±2.48	50.49 ±7.04*	0.00606 ±O.000436* * *	0.153 ±0.0123*
CR (30mg/kg) Col(3µg/10µl)	35.92 ±2.67* *	2.50 ±0.22* *	21.95 ±O. 986 *	64.75 ±14.97**	0.00321 ±0.000135 * * *	0.205 ±0.0150* * *

Table 1.5: CR effect on memory performance in the Morris water maze paradigm for aluminium chloride treated rats

Group	**Session 1**	**Session 2**	**Session 3**
Normal Control	28.50 ± 6.12	16.61 ± 0.60	10.78 ± 1.54* *
Aluminium Chloride (100 mg/kg)	59.94 ± 3.61	73.28 ± 4.92	80.50 ± 3.56**
CR (30mg/kg)	36.78 ± 1.91	18.89 ± 3.39**	17.72 ± 3.46***
CR (15 mg/kg) + AICl3(100mg/kg)	37.78 ± 4.03	24.0 ± 2.61 **	23.83 ± 1.39**
CR (30 mg/kg) + AICl3(100mg/kg)	39.50 ± 5.75	27.22 ± 1.58	35.28 ± 2.94

Table 1.6: CR effect on ALCL, induced memory impairment in Passive avoidance test

Group	**Acquisition trial**	**Retention trial 1**	**Retention trial 2**
Normal Control	17.00 ± 3.98	472.2 ±60.79***	548.7±23.94* * *
AIC13 (100 mg/kg)	31.83 ± 4.26	21.33 ± 0.84*	20.17 ± 1.35*
CR (30mg/kg)	70.67 + 21.70	450.8 +70.47* * *	565.0 ±22,57* * *
CR (15mg/kg) +AIC13 (100 mg/kg)	1 9.00 ± 4.45	138.3 ±23 73 *	144,4 ±44.73 *
CR (30mg/kg) +AIC13 (100 mg/kg)	24.50 ± 6.04	342.7 ±76.49* *	5372±52.51 ***

Table 1.7: Effect of AlCl and CR on Locomotor Activity

Group	**1st day**	**7thDAY**	**14th DAY**	**21 St DAY**	**28t DAY**	**35t DAY**	**42DAY**
Normal Control	178.0±14.63	162.8 ±12.3	1983 ±18.19	172.7±15.08	199.0 ±12.62	196.81±9.59	172.8 ±9.92
AIC13(100 mg/kg)	203.5 ±33.64	192.5 ±27.38	164.7 ±21.77	152.7 ±20.17	105.04±23.92	137.3±31.29	121.5±19.85
CR (30 mg/kg)	270.5 ±42.28	260.8 ±32.11	269.5 ±29.25	260.7±24.59	225.8 ±40.51	198.0 ±5.42	263.3±36.63
CR (15 mg/kg) +AIC13(100mg/kg)	230.7±25.43	214.5 ±24.29	162.8 ±20.25	179.8 ±7.36	166.7 ±9.01	166.8 ±18.5	226.2±14.84
CR (30 mg/kg) +AlCl3 (100 mg/kg)	118±21.03	123.8 ±21.29	121.3 ±16.95	125.3 ±20.05	124.2 ±31.25	132.8±24.42	135.3±30.84

Table 1.8: CR effect on oxidant- antioxidant level in rat's brain. Outcomes were expressed as mean ± S.E.M, n = 6 and examined by one-way ANOVA followed by Dunnet's test. *P <0.05, *P<0.01, p <0.001, when High dose and Low dose groups are compared with ALCL, 100 mg/kg. #P< 0.05, ##P< 0.01, ###P< 0.001 when group ALCL, 100 mg/kg compared with Normal control group.

Groups	**GSH**	**MDA**	**NITRIC OXIDE**	**SOD**	**ACHE**	**CATALASE**
Normal control	53.30 ±4.47	0.420 ±0.03	0.42±0.026	0.200 ±0.006	0,00024±0.0000019	0.200±0.006
AIC13 (100mgkg)	20.99±3.42###	1.55 ±O. 16###	1.554±0. 165#4#	0.036 ±0.0044###	0.00762±0.000380###	0,036±0.004
CR (30mg/kg)	40.95±3.80	0.703 ±0.05	0.70 ±0.05	O, 179±O.OIO	0.00048±0.000105	O. 179±0,011
CR (15mg/kg) + AIC13 (100 mg/kg)	28.49±2.01	1.04±O.06* *	1.04 ±0.063**	0099 ±0.011*	0.00520±0,000394*	0,084±0.005*
CR (30mg/kg) + AIC13 (100mg/kg)	34.99±3.01	0.80±0.02***	0.80±0.028***	0.123±0.025**	0,00103 ±0.000167***	0.130±0.020***

5. CONCLUSION

The research demonstrates that CR protects against neurodegenerative behavioral and biochemical alterations brought on by Colchicine (Col) and aluminium chloride (ALCL). Nevertheless, it can also be utilized as an antioxidant, anti-edema agent, and anti-cancer agent. Due to its ability to scavenge free radicals, it is also effective in treating Parkinson's disease and Huntington's disease. Pharmacological treatments like CR, which can protect neurons from oxidative damage by scavenging free radicals, preserving antioxidant homeostasis, and/or inhibiting lipid hydroperoxides and protein oxidation, may offer useful therapeutic potentials for the prevention or treatment of neurodegenerative diseases brought on by ALCL-induced neurotoxicity. For the prevention and treatment of Al-induced oxidative brain damage, CR's neuroprotective activity may be useful.

The current study also demonstrated that CR therapy improved mice's Col-induced memory impairment. CR may also have positive benefits on the brain by reducing oxidative stress and cholinergic dysfunction. CR may thus be an effective drug for the management of conditions associated with oxidative stress and cognitive decline.

REFERENCES

1. Hardman JG, Limbird LE, Gilmann AG. The pharmacological basis of therapeutics. Tata Mc Graw Hill. 2001;10: 549-556.
2. Mohsen K, Faezeh H. Effects of Active Constituents of Crocus sativus L, CR on Sporocin-Induced Model of Sporadic Alzheimer's Disease in Male Rats. Irani Biomedical Journal. 2010; 14:59-65.
3. Nair SC, Kurumboor SK, Hasegawa JH, Saffron chemoprevention in biology and medicine a review. Cancer Biother 1995; 10: 257-264.
4. Shen LH, Li HY, Shang HX. Synthesis and cytotoxic evaluation of new Col derivatives bearing 1,3,4-thiadiazole moieties. Chinese chemical letters. 2013; 24: 299-302.
5. Sherki YG, Melamed E, Offen D. "Oxidative stress induced neurodegenerative diseases: the need for anti-oxidant that penetrate the blood brain barrier Neuropharmacology 2001: 40(8):959-975.
6. Shimi S, Takashi O, Paopong L. CR suppresses tumor necrosis factor-a-induced cell death of neuronally differentiated PC-12 cells. Life Sciences. 2001; 69(24): 2887-2898.

7. Soedan S, Ochiaia T, Paopongb L. CR suppresses tumor necrosis factor-a induced cell death of neuronally differentiated PC-12 cells. Life Sciences. 2001; 69: 2887-2898.
8. Squire LR, Zola SM. Structure and function of declarative and non-declarative memory systems. PNAS. 1996; 93: 13515-13522.
9. Coulibaly AY, Sombie PA, Tibiri A, Kiendrebeogo M, Compaore MM, Nacoulma OG. Protective effect of Scoparia dulcis on brain and erythrocytes. Current Research Journal of Biological Sciences. 2011 May 5;3(3):254-61.
10. Husein AAM, Farath KM, Mohamed MG. Effects of chronic aluminum administration on brain and liver enzyme activities and essential elements concentration in rats: protective role of vitamin A and riboflavin. 2012: 1-21.
11. Atienzar F, Desor D, Burnel D, Keller JM, Lehr P, Vasseur P. Effect of aluminum on superoxide dismutase activity in the adult rat brain. Biological trace element research. 1998 Oct; 65:19-30.
12. Jin, CH, Wu SW, Zhou P, Liu QF, Lu XB, Shi LD and Cai Y. Effect of aluminum on Ca^{2+} concentration and expression of phospholipase C and NMDA receptor α genes in hippocampus of weaning rats as well as their neural behavior through subchronic exposure. Zhonghua lao Dong wei Sheng zhi ye Bing za zhi= Zhonghua Laodong Weisheng Zhiyebing Zazhi= Chinese Journal of Industrial Hygiene and Occupational Diseases. 2010; 28(9): 648-651.
13. Ingole SR, Rajput SK, Sharma S. Cognition Enhancers: Current strategies and future perspectives. Current research and information on Pharmaceutical sciences. 2008; 9:42-48.
14. Yuan CY, Lee YJ, Hsu GS. Aluminum alters NMDA receptor IA and 2A/B expression on neonatal hippocampal neurons in rats. Journal of Biomedical Science 2011; (18): 81.
15. Abdullaev FI. Biological effects of saffron. BioFactors (Oxford, England). 1993 May 1;4(2):83-6.
16. Caballero-Ortega H, Riverón-Negrete L, Pereda-Miranda R, Rivera-Luna R, Hernández M, Pérez-López I, Espinosa-Aguirre JJ. In vitro evaluation of the chemopreventive potential of saffron. Revista de investigacion clinica; organo del Hospital de Enfermedades de la Nutricion. 2002 Sep 1;54(5):430-6.
17. Hoseinzadeh H, Karimi Gh, Niapoor M. Antidepressant effects of Crocus sativus stigma extracts and its constituents, CR and safranal, in mice. Acta Hort (ISHS) 2004; 650: 435-445 .
18. Chryssanthi DG, Lamari FN, Iatrou G, Pylara A, Karamanos NK, Cordopatis P. Inhibition of breast cancer cell proliferation by style constituents of different Crocus species. Anticancer research. 2007 Jan 1;27(1A):357-62.
19. Escribano J, Alonso GL, Coca-Prados M, Fernández JA. CR, safranal and picroCR from saffron (Crocus sativus L.) inhibit the growth of human cancer cells in vitro. Cancer letters. 1996 Feb 27;100(1-2):23-30.
20. Papandreou MA, Kanakis CD, Polissiou MG. Inhibitory activity on amyloid-beta aggregation and antioxidant properties of Crocus sativus stigmas extract and its CR constituents. J Agric Food Chem 2006; 54: 8762-80.
21. Premkumar K. Abraham SK, Santhiya S, Tet al. Inhibition of genotoxicity by saffron (Crocus sativus L.) in mice. Drug Chem Toxicol 2001; 24:421-428.
22. Premkumar K, Abraham SK, Santhiya ST. Protective effects of saffron (Crocus sativus L.) on genotoxin-induced oxidative stress in Swiss albino mice. Phytother Res 2003; 17:614-617.
23. Nam KN, Park YM, Jung HJ. Anti-inflammatory effects of CR and crocetin in rat brain microglial cells. Eur J Pharmacol 2010; 648: 1-3.
24. Ochini T, Shimeno H, Mishima K. Protective effects of carotenoids from saffron on neuronal injury in vitro and in vivo. Biochim Biophys Acta. 2007;1770: 578-584.
25. Ochini T, Shimeno H, Mishima K. Protective effects of carotenoids from saffron on neuronal injury in vitro and in vivo. Biochim Biophys Acta. 2007; 1770(4):578-84
26. Abdullaev JF, Caballero-Ortega H, Riverón-Negrete L. "In vitro evaluation of thedemopreventive potential of saffron. Rev. Invest. Clin, 2002; 54.
27. Hosseinzadeh H, Ziace T, Sadeghi A. The effect of saffron, Crocus sativus stigma, extract and its constituents, safranal and CR on sexual behaviors in normal male rats. Phytomedicine 2008;15: 491-5.
28. El-Beshbishy HA, Hassan MH, Aly HA, Doghish AS, Alghaithy AA. CR "saffron" protects against beryllium chloride toxicity in rats through diminution of oxidative stress and enhancing gene expression of antioxidant enzymes. Ecotoxicology and environmental safety. 2012 Sep 1; 83:47-54.
29. Hoshyar R, Bathaie SZ, Sadeghizadeh M. CR triggers the apoptosis through increasing the Bax/Bcl-2 ratio and caspase activation in human gastric adenocarcinoma, AGS, cells. DNA and cell biology. 2013 Feb 1;32(2):50-7.
30. Zhang YX, Sugiura M, Saito H. Acute effects of Crocus sativus L. on passive avoidance performance in mice. Biol Pharmacol Bull 1994; 17:217-221.

31. Abe K, Sugiura M, Yamaguchi S, Shoyama Y, Saito H. Saffron extract prevents acetaldehyde-induced inhibition of long-term potentiation in the rat dentate gyrus in vivo. Brain Research. 1999 Dec 18;851(1-2):287-9.
32. Abdullaev FI. Cancer chemopreventive and tumoricidal properties of saffron (Crocus sativus L.). Experimental biology and medicine. 2002 Jan;227(1):20-5.
33. Hosseinzadeh H, Younesi HM. Antinociceptive and anti-inflammatory effects of Crocus sativus L. stigma and petal extracts in mice. BMC Pharmacol 2002; 2:1-8.
34. Jain KK. The Handbook of Neuroprotection. Springer Science 2011:25-28
35. Qi Y, Chen L, Zhang L. CR prevents retinal ischaemia/reperfusion injury-induced apoptosis in retinal ganglion cells through the PI3K/AKT signalling pathway. Exp Eye Res. 2013; 107: 44-51.
36. Rahani Foroutagheh M, Hamidoghli Y, Mohajeri SA. Effect of split foliar fertilisation on the quality and quantity of active constituents in saffron (Crocus sativus L.). J Sci Food Agric 2013.
37. Kulkami SK. Hand book of experimental pharmacology. Vallabh Prakashan 1999;117-119:135-136.
38. Tamaddonfard E, Farshid AA, Ahmadian E, Hamidhoseyni A. CR enhanced functional recovery after sciatic nerve crush injury in rats. Iranian Journal of Basic Medical Sciences. 2013 Jan 1;16(1):83-90.
39. Tamaddonfard E, Farshid AA, Asri-Rezaee S, Javadi S, Khosravi V, Rahman B, Mirfakhraee Z. CR improved learning and memory impairments in streptozotocin-induced diabetic rats. Iranian journal of basic medical sciences. 2013 Jan;16(1):91.
40. Patil S, Srinivas S, Jadhav J. Evaluation of CR and curcumin affinity on mushroom tynsinuse using Surface Plasmon Resonance. International Journal of Biological Macromolecules 2014; 65:163-166.
41. Ochiai T, Ohno S, Sooda S. CR prevents the death of rat pheochromyctoma (PC-12) cells by its antioxidant effects stronger than those of a-tocopherol. Neuroscience Letters. 2004;362(1): 61-64.
42. Vogel GH Drug discovery and Evaluation" Pharmacological assays. 2 edition, Springer science 2002:632.
43. Thiperwamy BS, Nagakannan P. Protective Effect of Emblin against Transient Global Ischemia induced brain damaged Neurofar Res. 2011: 379-386.
44. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology. 1961 Jul 1;7(2):88-95.
45. Totora GJ, Derrickson BH. "Principles of anatomy and physiology", 12" edition, vol 1; 2009:495-540.
46. Verstraeten SV Aimo L, Oteiza et al. Aluminium and lead: molecular mechanisms of brain toxicity Arch Taxicol 2008; 82(11):789-802.
47. Waugh A, Grant A. "Anatomy and Physiology in Health and Illness", Churchill Livingstone. 11 ed 2012: 148-154.
48. Zheng YQ, Liu JX, Wang JN. Effects of CR on reperfusion-induced oxidative nitrative injury to cerebral micro vessels after global cerebral ischemia. Brain Research. 2007; 1138:86-94.
49. Naghizadeh B, Mansouri MT, Ghorbanzadeh B, Farbood Y, Sarkaki A. Protective effects of oral CR against intracerebroventricular streptozotocin-induced spatial memory deficit and oxidative stress in rats. Phytomedicine. 2013 Apr 15;20(6):537-42.
50. Razavi BM, Hosseinzadeh H, Movassaghi AR, Imenshahidi M, Abnous K. Protective effect of CR on diazinon induced cardiotoxicity in rats in subchronic exposure. Chemico-biological interactions. 2013 May 25;203(3):547-55.
51. Lowry OH, Rosebrough NJ, A.Lewis Farr. Protein measurement with folin phenol reagent. J Biolchem 1991; 193: 265-275.
52. Jahanbakhsh Z, Rasoulian B, Jafari M. Protective effect of CR against reperfusion-induced cardiac arrhythmias in anaesthetized rats. EXCLI Journal 2012; 11: 20-29.
53. Zarei Jaliani H, Riazi GH, Ghaffari SM. The effect of the crocus sativus L. Carotenoid, CR, on the polymerization of microtubules, in vitro. Iran J Basic Med Sci. 2013; 16(1): 101-7.
54. Zumkley H. Bertram HP, Lison A. Al, Zn and Cu concentrations in plasma in chronic renal insufficiency. Clin Nephrol 1979; 12: 18-21.
55. Hariri AT, Moallem SA, Mahmoudi M, Hosseinzadeh H. The effect of CR and safranal, constituents of saffron, against subacute effect of diazinon on hematological and genotoxicity indices in rats. Phytomedicine. 2011 Apr 15;18(6):499-504.
56. Akhtari K, Hassanzadeh K, Fakhraci B. A density functional theory study of the reactivity descriptors and antioxidant behavior of CR Elsevier. 2013; 1013: 123-129.
57. Sugiura M, Shoyama Y, Saito Y. CR (crocetin di-gentiobiose ester) prevents the inhibitory effect of ethanol on long-term potentiation in the dentate gyrus in vivo. JPET 1994; 271: 703-707.

58. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. Journal of biological Chemistry. 1974 Nov 25;249(22):7130-9.
59. Kumar A. Dogra S. Neuroprotective effect of carvedilol, an adrenergic antagonist against Cols induced cognitive impairment and oxidative damage in rat. Pharmacology, Biochemistry and Behavior 2009;92:25-31.
60. Riyaz A. Dar, Pradeep K. Brahman, N. Khurana. Evaluation of antioxidant activity of CR, podophyllotoxin and kaempferol by chemical, biochemical and electrochemical assays. Arabian Journal Chemistry 2013.
61. Saladin KS. "Human anatomy". Tata Mc Graw Hill, 3 editions. 2011: 398-414.
62. Schipita AHV, Olanow CW. "Neuroprotection in Parkinson's disease: Mysteries, myths and misconceptions". JAMA. 2004; 291: 358-364.
63. Melo A, Monteiro L, Lima RM, de Oliveira DM, de Cerqueira MD, El-Bachá RS. Oxidative stress in neurodegenerative diseases: mechanisms and therapeutic perspectives. Oxidative medicine and cellular longevity. 2011 Oct;2011.
64. Kono Y. Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys 1978: 189–195.
65. Lari P. Abnous K. Imenshahidi M. Evaluation of diazinon-induced hepatotoxicity and protective effects of CR. Toxicol Ind Health. 2013; 13.
66. Lan P, Rashedinia M, Abnous K. CR Improves Lipid Dysregulation in Subacute Diazinon Exposure through ERK1/2 Pathway in Rat Liver. Drug Res. 2013.
67. Jnaneshwari S, Hemshekhar M, Santhosh MS, Sunitha K, Thushara R, Thirunavukkarasu C, Kemparaju K, Girish KS. CR, a dietary colorant mitigates cyclophosphamide-induced organ toxicity by modulating antioxidant status and inflammatory cytokines. Journal of Pharmacy and Pharmacology. 2013 Apr;65(4):604-14.
68. Luck H. Catalase, in Methods of enzymatic analysis edited by Bermeyer, H.W (Academic press, New York) See 3;1963:885.
69. Kyong Nyon Nam, Young-Min Park, Hoon-Ji Jung. Anti-inflammatory effects of CR and crocetin in rat brain microglial cells. European Journal of Pharmacology 2010;648(1-3): 110-116
70. Ghahghaei A, Bathaie SZ, Kheirkhah H, Bahraminejad E. The protective effect of CR on the amyloid fibril formation of Aβ42 peptide in vitro. Cellular & molecular biology letters. 2013 Sep; 18:328-39.
71. Gupta YK, Kumar VMH. "Intracerebroventricular administration of Col produces cognitive impairment associated with oxidative stress in rats". Pharmacology, Biochemistry and Behaviour 2002; 73:565-571.
72. Rabani-Foroutaghich M, Hamidoghli Y, Mohajeri SA. Effect of split foliar fertilisation on the quality god quantity of active constituents in saffron (Crocus sativus L.) Journal of Stroke and Cerebrovascular Der 2014; 23(1) :106-113.
73. Razavi M, Hosseinzadeh H. Abnous K. CR restores hypotensive effect of subchronic administration of diazinon in rats. Iran J Basic Med Sci 2013; 16(1): 64-72.