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Neuro-Protective Impact of Rutin Against Methionine-Induced Hyperhomocysteinemia in Rat Model

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ABSTRACT

Hyperhomocysteinemia HHcy, a pathological condition characterized by an increase in plasma concentration of total homocysteine Hcy, and recognized as a risk factor for several diseases. Rutin is a flavonoid component of plants that possess several pharmacological activities. This study aimed to evaluate the protective effect of supplementing high and low doses of Rutin on the experimentally induced mild Hyperhomocysteinemia. Sixty adult male Wister rats were used, divided into 6 groups as follow: G1: Control ,G2 : Hyperhomocysteinemia, induced by dietary feeding of 10 g/kg of L-methionine ; G3: Rutin (Low dose) rats received Rutin orally (25mg /kg bw/day); G4 : Rutin (High dose) rats received Rutin orally (200 mg/kg bw/day);G5: HHcy+Rutin (Low dose):Group 6: HHcv+Rutin (High dose). The study evaluated serum levels of homocysteine, cystathionine B-synthase, brain indices neurotransmitters, 8-Hydroxy-2-deoxyguanosine, oxidative biomarkers , DNA fragmentation and histological assessments. Results showed that. Methionine increased levels of serum homocysteine, LDH and 8- Hydroxy-2-deoxyguanosine, and reduced serum levels of cystathionine B-synthase, dopamine, serotonin, norepinephrine and gamma amino butyric acid. Also, caused dramatic disturbance on levels of oxidative stress biomarkers in brain homogenates. These findings were further supported by the histopathological evaluations and DNA fragmentation. Moreover, results suggested that, consumption of Rutin either in high or low dose posses a protective effect against neurological disorders, induced by methionine. Comparing treatments revealed that, high dose of Rutin was more potent than low one.

INTRODUCTION

Homocysteine Hcy, is a non-protein sulfur amino acid generated after supplementing methionine, either from diet or endogenous degradation of proteins ¹. Level of Hcy inside cells was controlled by two metabolic pathways: the re-methylation pathway, and the trans-sulfuration pathway (which regulated by the cystathionine βsynthase enzyme)². Raised levels of Hcy (> 12–15 μ M) induced hyperhomocysteinemia HHcy, which classified as mild (13-30 μ M), moderate (31-100 μ M), and severe (> 100 µM) ³. An increased frequency of mild and moderate HHcy occurred in some cases including feeding methionine-rich diet, shortage of vitamin B complex, genetic deficiencies, renal failure, and medicament usage ⁴. Lifestyle disorders, such as excessive coffee or alcohol intake, cigarette smoking and physical inactivity, play a vital role in modulating Hcy's plasma levels 5. Although, diets enriched in vegetables , fruit, and bread can result in lowering plasma levels of Hcy 6. There are no clinically observable features on physical examination that have been associated with homocysteinemia. However, serum levels of homocysteine are readily available and are standardized nationally ⁷. The brain is susceptible to increased levels of Hcy in blood, because it lacks two major metabolic pathways for its elimination: betaine remethylation and trans-sulfuration⁸. The disturbance in antioxidant balance in brain cells , leads to development of neurologic diseases, as brain tissue is very sensitive to oxidative stress⁴.

Flavonoids are among the strongest groups of phytochemicals found in herbs, vegetables , nuts, berries, tea, wine, spices and whole grains ⁸. More than 4,000 different forms of flavonoids, including anthocyanidins, flavonols, flavonoids, flavones, isoflavones and catechins have been identified in different categories ⁹.

Keywords: Rutin - Methionine - Hyperhomocysteinemia

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Figure (1): Chemical Structure of Rutin ⁹.

Rutin RT is a flavonoidal member of the phytochemicals with a base skeleton of 3,4,5,7-terahydroxyflurone-3-Drutinoside (Fig-1). Chemically it is a glycosidic compound , composed of flavonolic aglycone quercetin along with disaccharide rutinose, its name initiates from the plant Ruta graveolens 9. It is naturally found in passion flower, buckwheat, green asparagus, apples, tea, coffee, cocoa and cola ¹⁰. Rutin has numerous pharmacological characteristics including, antioxidant, anti-inflammatory, cytoprotective, vaso-protective, anticarcinogenic, neuroprotective and cardioprotective activities ¹¹.This study aimed to evaluate the protective effect of supplementing high and low doses of Rutin on the experimentally induced hyperhomocysteinemia. Materials and method:

Chemicals:

Pure Rutin powder was purchased from Thompson ® products, USA, Methionine (Molecular Weight: 149.21) of the highest grade obtained from Sigma-Aldrich (St. Louis, MO, USA),

Animals:

Sixty adult male Albino rats weighing 200 ± 10 g, supplied from the Breading Unit of Animal Reproduction Research Institute (Giza, Egypt). The rats were housed individually in stainless cages in an air-conditioned room maintained at 24°C with a 12-h light– dark cycle. After acclimation

period, the animals were left for randomly assigned into six experimental groups (10 rats /group) which were organized as follows:

Group 1: Control untreated rats, fed AIN-93G diet ¹².

Group 2 : Hyperhomocysteinemia, Mild HHcy was induced by feeding rats AIN-93G diet supplemented with 10 g/kg of L-methionine for 21 days , according to Zhang et al., 13 .

Group 3: Rutin (Low dose) normal rats received Rutin in oral dose (25mg /kg bw/day) dissolved in distilled water for 21 days according to Becho et al., ¹⁴.

Group4 : Rutin (High dose) normal rats received Rutin in oral dose (200 mg/kg bw/day) dissolved in distilled water for 21 days according to Tanko et al., ¹⁵.

Group 5: Hyperhomocysteinemia + Rutin (Low dose) after induction of HHcy, rats received Rutin orally in oral dose (25mg /kg bw/day) dissolved in distilled water for 21 days.

Group 6: Hyperhomocysteinemia + Rutin (High dose), after induction of hHcy, rats received Rutin oral dose (200 mg/kg bw/day) dissolved in distilled water for 21 days.

At the end of the experimental duration , rats were fasted overnight. The next day, rats were anesthetized by general volatile anesthesia using ether. Blood samples were collected by capillary micro-tubes from retro-orbital venous plexus and separated by centrifugation at 3000 rpm for 15 minutes, kept at -20 C for biochemical analysis. Brain was removed immediately by carefully fracturing the skull from the foramen magnum in the occipital region to the back of the skull. Brains were removed, weighed and immediately washed with ice-cold saline solution and stored at -80 °C till biochemical and histological investigations.

Preparation of brain homogenates

Each brain tissue sample was finely minced and homogenized in 50 mM phosphate buffer, pH 7.4, then centrifuged at 5000 ×g for 15 min at 4 ° C (Beckman Refrigerated Ultracentrifuge). Homogenates and supernatant were used for the biochemical assays.

Biochemical evaluations in serum

Serum Homocysteine, cystathionine B-synthase , dopamine and serotonin levels were determined by Enzyme Linked Immunosorbent Assay ELIZA using kits of (abcam, USA). Norepinephrine was determined calorimetrically by kits of (Novus Biologicals,USA). Lactate dehydrogenase LDH and Gama Amino Butyric Acid were established using (ELISA) kits produced by (lifespan Biosciences, USA).

Colorimetric determination of Deoxyribonucleic acid DNA fragmentation of brain tissues

DNA fragmentation was measured using diphenylamine DPA technique ¹⁶. About 0.4 mL of lysis buffer (10 mmol/L Tris, 1 mmol/L EDTA, pH 7.5) was added to brain homogenate and centrifuged to isolate the intact chromatin. The fragmented DNA, in the supernatant, was transferred to a distinct microfuge tube, and both supernatant then pellets were precipitated overnight in 12.5% trichloroacetic acid TCA. DNA precipitates were hydrolysed by heating in 5% TCA. For quantification of fragmented DNA, in brief, 0.16 mL of DPA reagent (0.15 g DPA, 0.15 mL H₂SO₄, and 0.05 mL acetaldehyde per 10 mL glacial acetic acid) was added to each tube and the absorbance was measured at 570 nm.

Biochemical evaluations in brain tissue homogenate

Brain tissue levels of 8-Hydroxy-2-deoxyguanosine and Protein carbonyl were determined by Enzyme Linked Immunosorbent Assay ELIZA using kits of (abcam, USA). While , Brain tissue levels of Glutamine synthase activity, Xanthine oxidase XO and superoxide dismutase SOD were established using (ELISA) produced by (lifespan Biosciences, USA).

Brain Tissues Histology

Whole brain tissues were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sections were made at thickness of 5 μ m, and stained with haematoxylin and eosin (H&E). The obtained slides were evaluated under light microscopy.

Statistical Analysis

Statistical analysis was performed SPSS program for windows, (version 20) (SPSS Inc., Chicago, IL, USA). Data were expressed as mean +/- standard deviation. The difference between groups was made using One Way ANOVA (LSD) test. *P*-value <0.05 was recognized as statistically significant

RESULTS

Table (1): Effect of different treatments on brain weight, serum level of homocysteine and cystathionine B-synthase

| Groups | Brain weight (g) | Serum Homocysteine (µmol/l) | Cystathionine B- synthase (ng/ml) |
|-------------|-------------------------|-----------------------------------|---|
| Control | 2.04±0.02 | 11.7±1.54 | 21.6±1.54 |
| ННсу | 1.62±0.03ª | 24.5±1.64ª | 8.4±1.60ª |
| RT(L) | 2.05±0.02 ^b | 12.1±1.66 ^b | 21.1±2.13 ^b |
| RT(H) | 2.08±0.06 ^b | 12.2±2.04 ^b | 22.7±1.64 ^b |
| HHcy+RT(L) | 1.73±0.07 ^{ab} | 19.4±1.65 ^{ab} | 14.4±1.66 ^{ab} |
| HHcy +RT(H) | 1.92±0.02 ^{ab} | 14.6±1.42 ^{ab} | 16.7±2.74 ^{ab} |

Results were expressed as mean \pm SD , n=10

^a means ±SD are significant (*P*<0.05) compared with normal control group,

^b means \pm SD are significant (*P*<0.05) compared with HHcy group.

Results obtained in table (1) revealed that methionine induced hyperhomocysteinemia which manifested as increased levels of serum homocysteine and decreased levels of Cystathionine B-synthase in HHcy group as compared to control untreated rats. On the other hand, treatment using Rutin either low dose HHcy+RT(L) or high dose HHcy+RT(H), reduced Serum homocysteine and increased Cystathionine B-synthase significantly (P<0.05) as compared to HHcy group

| Table (2) : Effect of different treatments on serum levels of brain neurotransmitters and Lactate dehydro | ogenase |
|---|---------|
|---|---------|

| Group | Dopamine (ng/mL) | Serotonin (ng/mL) | Norepinephrine (ng/L) | Gama Amino Butyric Acid (nmol/L) | LDH (µmol/mg protein) |
|-----------------|-------------------------|------------------------|--------------------------|--|-----------------------------|
| Control | 1.85±0.03 | 13.15±7.3 | 160±3.64 | 130.43±4.5 | 2.78±0.65 |
| ННсу | 1.02±0.05ª | 7.20±1.5ª | 113±3.65ª | 82.64±3.5ª | 6.22±0.83ª |
| RT(L) | 1.82±0.02 ^b | 13.24±1.7 ^b | 158±5.64 ^b | 131.85±4.3 ^b | 2.77±0.72 ^b |
| RT(H) | 1.83±0.10 ^b | 13.10±2.2 ^b | 155±3.76 | 129.73±2.7 ^b | 2.69±0.83 |
| HHcy+ RT (L) | 1.43±0.05 ^{ab} | 9.12±1.8a ^b | 135±7.43 ^{ab} | 112.76±2.2 ^{ab} | 5.07±0.88 ^{ab} |
| HHcy+ RT (H) | 1.63±0.04 ^{ab} | 11.45 ± 2.6^{ab} | 151±4.56 ^{ab} | 98.65±5.8 ^{ab} | 3.88±0.41 ^{ab} |

Results were expressed as mean \pm SD , n=10

^a means ±SD are significant (*P*<0.05) compared with normal control group,

^b means \pm SD are significant (*P*<0.05) compared with HHcy group.

Results represented in table (2) showed altered values of brain indices and Lactate dehydrogenase in HHcy group compared with control. A significant reduction was noticed in serum levels of Dopamine, Serotonin, Norepinephrine and Gama Amino Butyric Acid with a parallel significant (P<0.05) increase in LDH serum level

in HHcy group compared to normal one. Similarly, treatment using Rutin either low dose as in HHcy+RT(L) rat group ,or high dose as in HHcy+RT(H) rat group improved these parameters when compared to HHcy group. Moreover, no significant (P<0.05) change detected in control rat groups administered Rutin either in low RT(L) or high dose RT(H) when compared to control untreated rats. Comparing results showed that, administering Rutin to hyperhomocysteinemic rats, HHcy+RT(H) has the most potent effect of inflammatory biomarkers.



Figure (2): Effect of different treatments on 8- Hydroxy-2-deoxyguanosine level in brain homogenates.

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Figure (3): Effect of different treatments on Protein carbonyl level in brain homogenates.



Figure (4): Effect of different treatments on Glutamine synthase activity in brain homogenates.



Figure (5): Effect of different treatments on Xanthine oxidase level in brain homogenates.

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Figure (6): Effect of different treatments on SOD activity in brain homogenates.

Figures (2-6), represented results of oxidative stress markers in brain tissue homogenate. Methionine induced hyperhomocysteinemia that caused a dramatic disturbance on levels of oxidative stress biomarkers in brain homogenates. A significant (P<0.05) increase was detected in 8- Hydroxy-2-deoxyguanosine, Protein carbonyl and Xanthine oxidase levels in HHcy rat group when compared to control one. In addition, significant (P<0.05) reduction was noticed in the level of Glutamine

synthase and SOD in HHcy rat group as compared to control rats. Whereas, supplementing Rutin either low dose as in HHcy+RT(L) rat group ,or high dose as in HHcy+RT(H) rat group mitigated the harmful effect of methionine. Comparing results obtained from different treatments revealed that , administering Rutin in high dose possesses the most potent action against oxidative stress.



As seen in Fig (7), comparing results showed that administering high dose of Rutin was able to augment DNA fragmentation on comparison to HHcy rat group.

Histological findings

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Figure (8) : Photomicrographs of rat brain histopathology in all treated groups, stained with haematoxylin and eosin x 40 (a) G1 ; Control group; show normal morphological appearance and layers arrangement, (b) G2; Hyperhomocysteinemic group, abnormal morphological structure, showed a distortion in the pyramidal cells, darkly stained nuclei appears (arrow),some cells are surrounded by clear haloes (c) G3; Rutin low dose normal rats; normal morphological appearance (d) G4; Rutin high dose normal rats; normal morphological appearance (e) G5; HHcy+Rutin (Low dose), Mild lesions of neural degeneration and lower pyramidal cell distortion (arrow) (f) G6 ; HHcy+Rutin (High dose), lower lesions of neural degeneration , a reduction in clear halos was noticed (arrow).

Results of rats brain histopathology represented a normal morphological appearance in control group G1(Fig-8a), G3; (Fig-8c) Rutin low dose normal rats and G4; (Fig-8d) Rutin high dose normal rats, as supplementing Rutin only either in high or low dose did not affect brain tissue of normal rats. Dietary treatment of methionine(Fig-8b) induced hyperhomocysteinemia characterized by tissue deterioration, abnormal morphological structure and a distortion in the pyramidal cells. On the other hand, treating hyperhomocysteinemic rats with either low dose (Fig-8e) or high dose (Fig-8f) of Rutin improved the tissue morphology as compared to hyperhomocysteinemic rat group.

This study was planned to evaluate the effect of administering of high or low doses of Rutin could reduce side effects in case of HHcy. For this purpose, the study evaluated serum levels of homocysteine, cystathionine Bsynthase, brain indices , oxidative biomarkers , DNA fragmentation and histological assessments were also Hyperhomocysteinemia studied. was induced experimentally in rats by supplementing rat diets with methionine. Previous studies 17 and 18 stated that, feeding a high-methionine diet (17 g/kg) for 4 weeks could develop HHcy. However, accumulation of Homocysteine following methionine administration occurs as follow: (1) activation of methionine to form S-adenosylmethionine (SAM) by adenosine triphosphate ATP. SAM is a methyl donor to different receptors and forms Sadenosylhomocysteine (SAH) as a by-product of this

DISCUSSION

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methyl reaction. (2) SAH is hydrolyzed to form homocysteine¹⁹. Homocysteine is produced in all cells; the brain lacks both cystathionine gamma lyase and betaine-homocysteine *S*-methyltransferase (BHMT) (responsible for converting Homocysteine to cystathionine and back to methionine respectively).

The noticed reduction in brain weight may due to the contribution of Hyperhomocysteinemia to the elevated risk of dysfunction in endothelial cells, which interfere with the blood supply to the brain, and the subsequent cognitive decline and low brain volume ¹. The experimentally induced HHcy in this study, was correlated with increase in serum levels of homocysteine, with a reduction in the enzyme level of cystathionine B-synthase. These results agreed with a previous works ^{20,21}.While, Rutin treatment markedly brought these parameters near normal levels, earlier reports showed that , Rutin had a neuroprotective efficacy ^{22,23}. A previous work by Annapurna et al., ²⁴ stated that Rutin is neuroprotective agent in cases of cerebral ischemia reperfusion injury.

Results showed that, supplementing high doses of Methionine reduced the serum levels of (Dopamine, Serotonin, Norepinephrine and Gama Amino Butyric Acid) as seen in HHcy group compared with control one. Methionine is involved in several methylation reactions in the brain including DNA, RNA, phospholipids and the synthesis of neurotransmitters such as serotonin, noradrenaline and dopamine. Increased concentrations of Hcy, resulted from diminished re-methylation to methionine, causes direct toxic effects to vascular endothelial cells and neuronal cells ²⁵. HHcy was also affecting the level of neurotransmitters such as serotonin, norepinephrine, epinephrine ²⁶.

A neurotransmitter is a chemical released from a nerve cell, that transmits an impulse to another nerve cell or an effector cell, such as a muscle cell 27 .This study results agreed with previous study 28 which stated that continued intraperitoneal administration of Hcy in mice leads to dopamine depletion. Furthermore, one study by Chandra et al., 29 showed that, Hcy-induced specific neuronal loss and dopamine depletion (50%) only after administration of Hcy. Moreover, another work by ³⁰ examined the possible neurotoxic effects of the increased homocysteine level on the dopaminergic system and indicated the potential of homocysteine to be toxic to the dopaminergic system. In addition, previous work by Jadavji et al., ³¹ revealed that, the increased levels of homocysteine have been linked to low levels of monoamines neurotransmitters (dopamine, serotonin, norepinephrine) levels in patients with depression.

GABA, is a major functionally inhibitory neurotransmitter in the nervous system which mediates numerous functional responses in non-neuronal tissue ³².This study results agreed with previous work by Tayagi et al., ³³ which stated that Hcy, was found to reduce GABA levels. Lactate dehydrogenase is a biomarker that commonly applied in toxicology to diagnose cell damage, while any variation in its activity level, detects metabolic changes in the affected tissues ³⁴.Our study results agreed with earlier study ³⁵ which displayed that administering methionine (1 g/kg, p.o.) for 30 days to increased homocysteine and LDH serum levels. Similar results of LDH were obtained ³⁶. Additionally, previous reports ^{37,38} documented the correlation between elevated plasma homocysteine level with increased level of LDH .

Results revealed that , Rutin administration in either high or low doses was claimed to attenuate the effect of methionine. A study by ³⁹ conveyed that, direct actions of dietary polyphenols depends on their metabolites, which exert neuroprotective effects after reaching the brain by crossing blood-brain barrier ³⁹. Our findings were in line with previous reports 40,41 which demonstrated that, Rutin in considered as antidepressant because it increase the availability of serotonin and norepinephrine in the synaptic cleft ⁴⁰. Another study by Javed et al., ²³ displayed a decrease in neuroinflammation in rat model of 'sporadic dementia of Alzheimer type' following Rutin administration. Similar study $^{\rm 42}$ on mice brain , showed that intravenous administration of Rutin kept memory deficits and enriched neurologic variations . Similarly, previous study 43 showed that, administering Rutin along with exercise improved cognitive defects that caused following feeding high fat diet in mice. Meanwhile, it was found that Rutin protects brain tissue of Wister male rats after exposure to gamma-irradiated 44. Preclinical researches revealed that neuroprotective effects of Rutin was related to its antioxidant and anti-inflammatory properties that manipulates intra- and extracellular mechanisms in central nervous system 45.

Findings of our study represented that, Methionine caused a dramatic disturbance in oxidative stress biomarkers levels in brain tissues homogenates. Hyperhomocysteinemia is associated with increased levels of oxidative stress and lipid peroxidation that affects neural cells and interfere with memory formation ⁴⁶. Oxidative stress is created during oxidation of the free thiol group of Hcy. However, the elevated production of ROS caused by Hcy may encourage subsequent oxidation of proteins, lipids and nucleic acids ⁴⁶. Additionally, Hcy causes oxidative stress by affecting cellular respiration, leading to oxidation of low density lipoproteins LDL ¹⁸.

The results of oxidative biomarkers in this study were consistent with those of Koilling et al., which found an increased levels of protein carbonyl products in brain tissue of HHcy rats compared with controls 47. Moreover ,Santos ⁴⁸ found that, HHcy reduced the activity of antioxidant enzymes (e.g., SOD and Glutamine synthase). Results of 8-OHdG were similar to previous works ^{49,21} who applied the immunofluorescence staining of 8-OHdG to detect the effect of HHcy , the results stated that amount of 8-OHdG positive cells were significantly higher in brain tissues of HHcy group compared with that in the control group. The reduction in SOD activity in the brain of rats during HHcy may be related to both neutralization of the superoxide radical that formed during HHcy and inactivation of the enzyme due to the oxidation of tyrosine amino acid residues which may enhance the toxic effects of free radicals in brain structures⁵⁰.

Comparing results represented that, using Rutin in treatment restored oxidative biomarkers levels near normal which agreed with previous studies ^{51, 52} which suggested that the antioxidant activity of Rutin accounts for its potential protective role in neurodegenerative. In addition , a previous work by Filip and co-workers ⁵³ stated that , Rutin treatment increase endogenous antioxidant enzymatic activities and enhanced the function of ischemia brain injury-related disorders. Our results also supported by Qu et al., ⁴¹ who showed that Rutin treatment markedly attenuated protein carbonyl level in rats. Besides, Rutin has neuroprotective effect against brain ischemia, as pretreatment with Rutin,

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reduced MDA, H_2O_2 and GSH in the hippocampus and frontal cortex in the middle cerebral artery occlusion (MCAO) in rats ⁵⁴. Additionally, Ramalingayyabet al., ⁵⁵ established that pretreatment with Rutin inhibited reduced CAT, GSH, and SOD levels in Wistar rats resulted from doxorubicin treatment.

On the other hand, Jiang et al., ⁵⁶ stated that Rutin intake improved antioxidant levels and inhibited the thiobarbituric acid reactive substances (TBARS) in the gastric mucosa.

Xanthine oxidase is an important enzyme of purine catabolism pathway and has been associated directly with increased ROS production and in pathogenesis of gout and indirectly in many pathological conditions like cancer, diabetes and metabolic syndrome 57. The study of Malik et al., 57 determined the capability of Rutin, to inhibit xanthine oxidase. They reported that Rutin and its derivatives exhibited remarkable activity in reducing xanthine oxidase activity comparable to the positive control. Glutamate, an excitatory amino acid, is one of the major neurotransmitter in the central nervous system ⁵⁸. In the brain, the conversion of glutamate to glutamine by glutamate synthetase (GS), that takes place within the astrocytes, denotes a key mechanism in excitatory neurotransmission regulation . Along with our results, the study of Swamy et al., 59 showed that treatment of epileptic rats with propolis (containing Rutin as the major flavonoid) resulted in significant improvement in GS activity and oxidative biomarkers in all brain regions. All these findings indicate that. Rutin is a neuroprotective polyphenol, and that, after systemic administration, Rutin passed through the blood-brain barrier to play its neuroprotective roles ²⁴.

According to studies on brain tissue, an increased levels of Hcy caused hypomethylation or hypermethylation of cellular DNA ⁴. Any change in this processes causes DNA fragmentation in DNA strands and increase in their signaling for errors, which may direct the cells to apoptosis and neuronal damage . Rutin was known to inhibit cancer cell growth by cell cycle arrest and/or apoptosis, along with inhibition of proliferation, angiogenesis, and/or metastasis in colorectal cell lines ⁶⁰. Similarly, in a study on potassium bromate-induced nephrotoxicity ,it was shown that Rutin demonstrated protective effects including a decrement in DNA fragmentation ⁶¹.

Conclusions

This study demonstrates that, methionine rich diet induced mild hyperhomocysteinemia correlated with elevated serum levels of serum homocysteine and LDH, reduced serum levels of Cystathionine B-synthase, Dopamine, Serotonin, Norepinephrine and Gama Amino Butyric Acid. Also, a dramatic disturbance on levels of 8-Hydroxy-2-deoxyguanosine ,oxidative stress biomarkers in brain homogenates, histopathological alteration and DNA fragmentation were also observed. The increased level of Homocysteine was implicated in increased oxidative stress, DNA damage, triggering excitotoxicity, all important mechanisms in neurodegeneration.Moreover, based on the results of our study, it can be assumed that Rutin supplementation either in high or low dose is effective in attenuating these parameters, indicating that Rutin rich sources may should be included in diet as natural source of neuroprotection . Lastly, light microscopic observations of brain tissue in HHcy group, showed tissue deterioration, abnormal

morphological structure and a distortion in the pyramidal cells, while treating rats with Rutin, even in high or low dose showed observable improvement , thus further supporting the role of Rutin as a neuroprotective agent in HHcy rats.

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