Hypolipidemic and Antioxidant Efficacy of Apigenin in Hydrogen Peroxide induced Oxidative Stress in Adult Male Rats

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Abstract

This study was conducted to assess the role of apigenin extracted from parsley seeds either in glycosidic (aqueous extract) or aglycone forms (organic solvent extract), comparing to butylated hydroxyl tolouene (BHT)in serum lipid profile and brain tissue peroxidation in H_2O_2 induced oxidative stress adult male rats.

The yield of crude flavonoids from parsley seeds was found to be approximately 2.65% and thin layer chromatography techniques confirmed that apigenin is the main flavonoid with RF similar to that of the standard apigenin.

Experimentally induction of oxidative stress in male rats by 0.75% H_2O_2 in drinking water for eight weeks showed a significant alterations in normal serum lipid profile manifested by significant elevation ($p<0.05$) in total cholesterol, Triacyle glycerol (TAG), Low density lipoprotein-Cholestrol (LDL-C) and Very low density lipoprotein-Cholestrol (VLDL-C) and a significant decrease in High density lipoprotein-Cholestrol (HDL-C) as compared to the control and BHT treated groups. On the other hand, daily oral administration of apigenin in a dose of 150 mg/Kg B.W. to H_2O_2 treated groups were caused a significant correction of the lipid profile parameters.

Examination of brain tissues of H_2O_2 and apigenin concurrent H_2O_2 treated rats showed a significant decrease ($p<0.05$) in brain tissue malondialdehyde (MDA) and significant elevation in catalase and cholinesterase activities in apigenin and BHT treated groups comparing with H_2O_2 treated and control groups.

Keywords: *Hypolipidemic, Antioxidant, Apigenin, Hydrogen Peroxide,, Oxidative Stress, Rats.*

Introduction

Recent attention has been given to the influence of dietary factors on health and mental well-being [(1)]. There are convincing evidences that the oxidative stress and reactive oxygen species (ROS) play an important role in the etiology and/or progression of a number of human diseases[(2)]. It is known that oxidative stress is associated with many diseases including neurodegenerative disorders.

Many factors contribute to the degeneration of neural cells, leading to functional deterioration of neural cells, leading to functional deterioration of neurons and neurodegenerative disorders. Hence, because of their high metabolic activities and low antioxidant defense capacities, neural cells in brain are more vulnerable to oxidative stress[(3)]. In addition, hydrogen peroxide is produced in β-amyloid (Aβ) aggregation, dopamine oxidation, and brain ischemia/reperfusion, AB aggregation is known to cause oxidative damage in neurons, including protein and lipid oxidation and DNA damage[(4)].

The main class of natural antioxidants is exerting cardioprotective, chemopreventive, and neuroprotective effects. The biological activities of flavonoids have been attributed to their antioxidant, anti-inflammatory, anticancer, neuroprotective and signaling properties[(1,5)]. A clear understanding of the mechanisms of action, as either antioxidants or signaling molecules, is crucial for the application of flavonoids as interventions in

neurodegeneration and as brain foods[6]. Some flavonoids (like apigenin) can also traverse the blood-brain barrier; hence they are promising candidates for intervention in neurodegeneration and as constituents in brain foods[7].

Apigenin (4, 5, 7 - trihydroxyflavone) is a dietary flavonoid commonly found in many fruits and vegetables[8]. It has been found that apigenin inhibits tumor growth and angiogenesis agent induced by different cancer cells[9].

However, the role of apigenin as antioxidant and in neuroprotection is not so clear therefor, this study was conducted in order to investigate the following:

1. Extraction of apigenin from parsley seeds and conformation of the structural components using (TLC) in comparison with standard apigenin.
2. Assessment the role of apigenin as either Glycoside and/or Aglycone in serum lipid profile and brain lipid peroxidation in oxidative stress male rats.

Materials and Method

Parsley seeds were obtained from commercial sources (Baghdad) and the vouchers specimen of the plant were deposited to be identified and authenticated at the National Herbarium of Iraq botany directorate in Abu-Ghraib under scientific name *petrselinumsativum* belongs to the family umbilifera. After cleaning and milling crushed seed was kept in dark and dry place.

The method of Harborne[10] modified by Al-Kawary[11] was used for the extraction of apigenin in aglycone form, while the method of Ikhan[12] was used for apigenin extraction in Glycoside form.

Thin layer chromatography on Silica gel type G aluminum plates (20 x 20 cm) at a thickness of 0.25 mm supplied from Fluka Company was used for the identification of apigenin either in Aglycone or Glycosidic form. Toluene: ethyl-acetate: acetic acid (36:12:5) was used as mobile phase [13] and UV detector to explore the spots on 254ηm.

Fifty adult male Albino Wister rats weights (250-300) gm, 10-12 weeks of age were randomly divided into five groups; Group (c), Rats of this group were allowed to ad libtium supply of drinking water and served as a negative control group. Group (T1), rats were allowed to 0.75% H_2O_2 in drinking water and served as a positive control group. Group (T2) rats, were allowed to 0.75% H_2O_2 plus daily oral administration of BHT, 25 mg/kg B.W.[14], using gavage needle. Group (T3) rats were allowed to 0.75% H_2O_2 + 150 mg/kg B.W of apigenin in glycosidic form. Group (T4) rats were allowed to 0.75% H_2O_2 + 150 mg/kg B.W of apigenin as Aglycone.

Blood and brain tissue samples were collected after four and eight weeks of experimental period, five animals from each group were sacrificed for blood and brain tissue analysis, immediately after blood collection, each animal was killed and the head was separated and quickly preserved in liquid nitrogen until analysis.

Serum samples were used for the measurement of total cholesterol according to[15] using Randox assay kit. Triacylglycerol using Biomerieux kit[16]. High density lipoprotein (HDL-C) measured enzymatically using linear enzymatic Kit (Linear chemicals, Barcelona, Spain).

Serum low density lipoprotein cholesterol (LDL-C) and VLDL-C were calculated according to[17].

Brain tissue samples were used for the measurement of cholinesterase activity according to[18], catalase activity according to[19] and malondialdehyde concentration[20].

Statistical analysis of data was performed of two way analysis of variance (ANOVA) using significant level of ($p<0.05$). Specific group differences were determined using least significant difference (LSD) as described by [21].

Results and Discussion

Parsley dry seed contains approximately 2.65% flavonoids (apigenin). The RF values of extracted apigenin in glycosidic form was 0.477, similar RF values under the same experimental conditions was recorded by [13], while the corresponding values for apigenin in aglycone form was 0.554. The higher RF values in aglycone form may attribute to the remove of sugar moiety from the molecule.

Oral administration of 0.75% H_2O_2 to male rats caused a case of hypercholesterolemia and hypertriacylglyceridema manifested by a significant $p<0.05$ elevation in TC, LDL-C, TAG (table 1, 2, 3, 4) and reduction in HDL-C (table 5), and this may reflect the potent oxidative effectiveness of H_2O_2 which caused an oxidative damage by free radical generation (superoxide anion, hydroxyl radical), led to a subsequent complication and the development of oxidative stress[22].

Administration of 0.75% H_2O_2 caused a significant increase in the circulating total cholesterol, LDL-C, VLDL-C and also in the ratio of TC: HDL-C and LDL-C: HDL-C. HDL-C inhibits the uptake of LDL-C by the arterial wall and facilitates the transport of cholesterol from peripheral tissue to the liver where they are catabolized. Then a decrease in plasma HDL-C leads to an elevation of LDL-C[23]. Besides, increment of TAG level in animals received H_2O_2 in the present study may be due to an increase in serum VLDL-C level which acts as a carrier for the TAG [24].

Anyway, hydrogen peroxide (0.75%) used in this study may be considered as exogenous stress factor in the production of ROS which media the damage of the cell structure including nucleic acid, proteins and lipids which lead to a consequent alteration in lipid profile and an elevation in total cholesterol [25, 26].

Moreover, the relationship between serum cortisol, adrenaline and lipid profile under different stress conditions have been documented by a number of workers [26, 27].

Adrenocorticotropic hormone (ACTH) stimulates the synthesis of adrenaline and cortisol precursors, and the role of these stress hormones in the production of more energy in the form of metabolic fuels, fatty acids and glucose is well documented these substances require the liver to produce and secrete more LDL, which is the main carrier of cholesterol in the blood and finally elevated cholesterol level [28, 29]. In this regard, the hypothesis postulated by [30] that oxidation stress brought about by the combination of excess liver iron and copper deficiency should be also considered since oxidative stress have a considerable role in hemolysis of RBC because of the depletion of antioxidant and particularly glutathione which play a major role in the protection of red blood cell membrane. The high iron level may increase both free radical formation and hyperlipidemia.

The hypolipidemic effect of flavonoid, (apigenin) may be attributed to the fact that cells respond to phytochemical through direct interaction with receptors or enzymes involved in signal transaction, or through modifying gene expressions [31, 32].

So, apigenin may limit cholesterol biosynthesis by inhibiting 3-hydroxy -3-methylglutaryl-CoA reductase (HMG-CoA reductase) or by enhancing the phosphorylation of HMG-CoA reductase indirectly thus minimize endogenous cholesterol production, and reduces apo B secretion in hepatocytes [33, 34] or, binding to cytoplasmic steroid receptor due to hydrophobicity of their aglycone portion. Moreover, flavonoids (apigenin) may intercalate between the DNA segments, leading to transcription of gene involved in lowering blood cholesterol[34, 35].

Oxidative status of brain tissues: Malondialdehyde (MDA) concentration, catalase and cholinesterase activity of brain tissue were considered in this study as a biomarker of H_2O_2 induced oxidative stress.

A significant ($p<0.05$) elevation in MDA was observed in H_2O_2 treated group after four and eight weeks of the treatment. Increase of MDA level may be due to an increase in free radicals production more than the ability of scavenging system, hence, elevated FRs cause a gradual cell injury by lipoxygenase enzyme which oxidized unsaturated fatty acids and subsequent production of excess MDA [36, 37].

Free radicals may also induce injury by induction of gene expression regulated by nuclear transcription factor and jun-NH_2-terminal kinase (JNK)a stress protein leading to cellular damage[38, 39].

After 4 and 8 weeks of treatment with BHT and apigenin (150 mg/kg B.W. as Glycoside or aglycone) no significant differences in MDA concentration in the brain tissue was recorded among the antioxidant treated groups. Improving the oxidative stress H_2O_2 treated groups was confirmed the scavenging and potent LPO inhibition capability of the apigenin and BHT. Apigenin may considered a part in indirect inhibition of nuclear transcription factor B, resulting in a decrease in the formation of,adhesion molecules, chemokines, pro-inflammatory cytokines, TNF, IL6 and IL8, as well as, binding to DNA strand [40].

Brain tissue catalase activity (Ku/100gm wet tissue) in H_2O_2 treated group, there was a significant decrease ($p<0.05$) during the experimental period, while no significant alterations was recorded in apigenin and BHT treated groups. Excessive H_2O_2 production with diminution of antioxidant enzyme and subsequent reaction of H_2O_2 with reduced iron to produce hydroxyl radical via Fenton reaction [41] has been postulated to cause elevation in ROS with depletion of antioxidant[42].

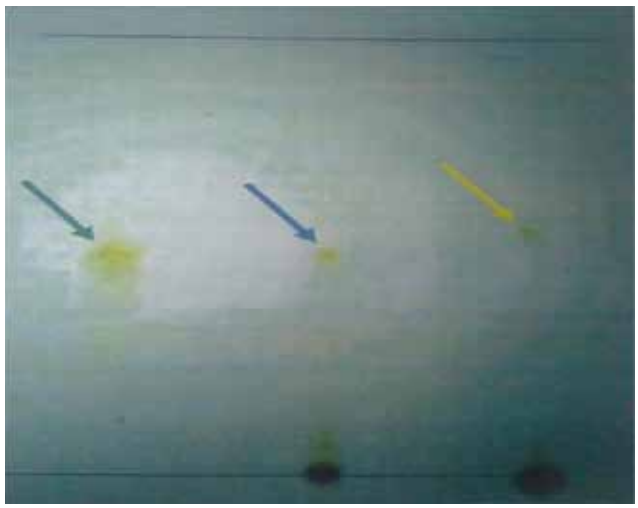
Besides, oxidative stress in the brain is associated with increased calcium ion concentration and increased mitochondrial demand leading to an increase in the

formation of ROS and RNS by disturbing respiratory chain and activation of series of enzyme like nitric oxide synthase and xanthine oxidase which stimulate ROS formation and antioxidant enzymes depletion [1].

On the other hand, the significant role of apigenin in ameliorating of antioxidant status may be attributed to its capability in inhibiting the activity of xanthine oxidase[43], lipoxygenase and cyclooxygenase enzymes[44].

Moreover, Hydrogen peroxide treated group also showed a significant decrease in brain tissue cholinesterase activity and this may be attributed to the role of H_2O_2 in the oxidation of the Trp_{432}, Trp435 and Met 436 residues in the active site of the enzyme resulting in conformational changes and loss of the physiological function[45].

However the result of this study confirmed that apigenin has no neuroprotective effect within the concentration of H_2O_2 used because acetylcholinestrase activity is H_2O_2 concentration dependent and inhibited at high H_2O_2 concentration (10^{-3} M) and activated at low concentration (10^{-6} M) [45] as well the dose of apigenin used may not effective to reactivate the enzyme, although the possible mechanisms underlying the neuroprotection of flavonoids against H_2O_2 induced oxidative damage in PC_{12} cells was recorded by [1].



Standard apigenin,
Parsley seed extracted apigenin (Glycoside)
Parsley seed extracted apigenin (Aglycone)

Figure 1: TLC plte (silica gel),

Table 1: Serum total cholesterol (TC) concentration (mmol/L) of rats exposed to oxidative stress via 0.75% hydrogen peroxide in drinking water at the periods xero, four and eight weeks in different groups of the experiment

Weeks / Groups	0	4 weeks	8 weeks
C	2.468±0.194 Aa	2.461±0.194 Aa	2.478±0.194 Ba
T1	2.468±0.194 Aa	2.566±0.205 Aab	2.94±0.172 Aa
T2	2.468±0.194 Aa	1.932±0.094 Bb	1.704±0.094 Cb
T3	2.468±0.194 Aa	1.853±0.088 Bb	1.756±0.066 Cb
T4	2.468±0.194 Aa	1.955±0.172 Bb	1.985±0.144 Cb

L. S. D. = 0.394

Table 2: Serum low density lipoprotein-Cholesterol (LDL-C) concentration (mmol/L) of rats exposed to oxidative stress via 0.75% hydrogen peroxide in drinking water at the periods zero, four and eight weeks in different groups of the experiment.

Weeks / Groups	0	4 weeks	8 weeks
C	1.191±0.033 Aa	1.194±0.033 Ba	1.197±0.033 Ba
T1	1.191±0.033 Ac	1.279±0.044 Ab	1.686±0.027 Aa
T2	1.191±0.033 Aa	0.72±0.022 Cb	0.45±0.022 Dc
T3	1.191±0.033 Aa	0.527±0.022 Db	0.501±0.022 Dc
T4	1.191±0.033 Aa	0.699±0.022 Cb	0.764±0.016 Cb

L.S.D.= 0.077

Table 3: Serum very low density lipoprotein-Cholesterol (VLDL-C) concentration (mmol/L) of rats exposed to oxidative stress via 0.75% hydrogen peroxide in drinking water at the periods zero, four and eight weeks in different groups of the experiment.

Weeks / Groups	0	4 weeks	8 weeks
C	0.580±0.011 Aa	0.583±0.011 ABa	0.489±0.011 Ba
T1	0.580±0.011 Ab	0.629±0.022 Aab	0.664±0.027 Aa
T2	0.580±0.011 Aa	0.629±0.022 Bab	0.471±0.016 Cb
T3	0.580±0.011 Aa	0.561±0.022 Ba	0.526±0.027 BCab
T4	0.580±0.011 Aa	0.551±0.027 Bab	0.505±0.016 Cb

L.S.D.= 0.066

Table 4: Serum triacylglycerol (TAG) concentration (mmol/L) of rats exposed to oxidative stress via 0.75% hydrogen peroxide in drinking water at the periods zero, four and eight weeks in different groups of the experiment.

Weeks / Groups	0	4 weeks	8 weeks
C	2.904±0.005 Ab	2.911±0.005 ABa	2.909±0.005 Bb
T1	2.904±0.005 Ab	3.148±0.116 Aab	3.324±0.133 Aa
T2	2.904±0.005 Aa	2.603±0.022 Cb	2.36±0.161 Cbc
T3	2.904±0.005 Aa	2.81±0.105 Ba	2.635±0.161 BCab
T4	2.904±0.005 Aa	2.76±0.105 BCab	2.528±0.88 Cb

L.S.D.= 0.288

Table 5: Serum high density lipoprotein-Cholesterol (HDL-C) concentration (mmol/L) of rats exposed to oxidative stress via 0.75% hydrogen peroxide in drinking water at the periods zero, four and eight weeks in different groups of the experiment.

Weeks / Groups	0	4 weeks	8 weeks
C	0.692±0.038 Aa	0.689±0.038 Aa	0.682±0.038 ABa
T1	0.692±0.038 Aa	0.657±0.027 Aa	0.578±0.027 Bb
T2	0.692±0.038 Aa	2.691±0.022 Aa	0.782±0.011 Aa
T3	0.692±0.038 Aa	0.728±0.044 Aa	0.764±0.161 Aa
T4	0.692±0.038 Aa	0.704±0.038 Aa	0.715±0.061 Aa

L.S.D.= 0.111

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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