Effect of Mulberry crud extract as antioxidant and antiatherogenic experimentally on rabbit

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Abstract

This study was conducted to explore the ability of mulberry (Morus albs L.) cold and bold crud equeous extract as antioxidant and antiatherogenic in rabbit after exposure to 0.5% H\textsubscript{2}O\textsubscript{2} in drinking water for 30 days.

The results illustrated capability of 0.5%H\textsubscript{2}O\textsubscript{2} to initiate oxidative stress via significant increase in malondialdihyde (MDA) level in heart and liver concomitant with significant decrees in Glutathione (GSH) in addition to decreas in high density lipoprotein-c HDL and increase in low density lipoprotein-c (LDL-c) in serum of treated rabbits. While rabbit treated with H\textsubscript{2}O\textsubscript{2} associated with mulberry leaves (cold and boild aqueous crud extract) showed a significant increase in GSH concentration of liver and heart tissue as well as increase in HDL-c, decrees in LDL-c and atherogenic index. Histologically, heart, aorta showed less reduction in lipid vacuol and proliferation of vascular smooth muscle cell in media toward intima, but it is not reach to normal picture in rabbit treated with mulberry (Morus albs L.) while liver sections showed some vacuolar degeneration.
Introduction

The imbalance between antioxidants (antioxidante defense) and increased free radicals production leading to oxidative damage is known as oxidative stress (1). An example of this process is oxidation of membrane bound lipids and lipoprotein known as lipid peroxidation (2). The peroxidation of cellular membrane lipids can lead to cell necrosis and is considered to be implicated in a number of pathophysiological conditions (3) as a type I diabetes mellitus and atherosclerosis. (Morus albas L.) (Moraceae) (MA) white mulberry has along history of medical use in Chinese medicine parts of plant are used as medicine. White mulberry is cultivated through leaves is the main food source for silk worms. Traditionally, the white mulberry plant has been used to treat weakness, fatigue, anemia, and preastare grayi in continence, tinnitus, dizziness and constipation in the elderly patient so far include analgesic, antiasthmatic, antirhaumatic, antitusive, as expectorant, hypotensive and brain tonic (4-8). The plant extraction have radical scavenging activity (9) Hyperglycemic and antioxidant pontential, hepatoprotective hypourecemic and cardioprotective action (10). Substances which attract the silk worms larvae to the leaves have been identified as citral linulyl acetale, linalol, terping, acitle, and acelate, hexenol, the first 3 being more effective. B-sitosterol (cazol in leaves) along with some sterols and a water soluloble substances it is the main factor which stimulate the biting action (11).

The amount of food eaten is controlled by the cencentration of B-sitosterol. Protein preparation from young mulberry leaves form an exacellent supplement to protein-deficient diet. Non –protein nitrogen accounts for Ca 22% of the total nitrogen in young leaves and Ca 14% in mature leaves – Amino acids identified in the free form are phenylalanine, leucine, valane, tyrosine, proline, alanine, glutamic acid, glycine, serine, arginine, aspartic acid, cystine, threonine, sacosine, gamma– amino butyric acid, pipercolic acid and S-hydroxy pipercolic acid. the leaves are good source of ascorbic acid, with 2-3 ml/g of which over 90% is present in the reduced form. They contain also vitamin B1, folic acid, folinic acid and vitamin D. Phytate phosphores account for 18.2% of total phosphores. Volatile constituents identified in steam-distillates of the leaves are n- butylaldehyde, isobutylaldenyale, valeraldehyal hexaldehyde, alpha-beta-hexenal acetone, methyl-ethylketone, methyl-hexyl-ketone, butyline, acetin, propionic and isobutyrlic acids, leaves also contain calcium malate, succinic and tartaric acids, xanthophylls and isoquercitrin (quercetin3-glucosid) and tannis, adenine, chorine, and trigonelline bases are present in young leaves. analysis of leaves give (dry weight basis). protein 14.0; EE, 6.8; NFE, 49.7; total ash 13.8; calcium (Cao, 2.74; and phosphores ($P^2O_5$) 0.45% (11). In this study we used $H_2O_2$ in 0.5% (12) to induce oxidative stress to answer the question whether M. albas L. leaves extract treatment reduce lipid peroxidation? also to examine the ability of M. albas L. as antioxidant and antiatherogenic effect.

Material and Methods

Male local breed rabbits (15) weighting between 750-850 gm were used. The animals were reared in wire cages feed diet and given tap water ad libitum, and housed in a constant (22-25°C) temperature environment with (12h.light and 12h.dark cycle). Animals were divided into five groups of three rabbits each.Group1(GI) received a regular rabbit diet and maintained as a control group; Group II rabbits subjected to experimentally induced oxidative stress by the ad libitum supply of drinking water containing 0.5% $H_2O_2$ (V/V) [degussa AG merl Rheinfelden, Belgium] was prepared daily over entire 30 days period of the experiment after the end of 30 days of $H_2O_2$
treated animals were selected randomly to serve as the *Morus albas* L. aqueous extract treated group (GII) animal of this group received extract of *M. albas* L. for seven days. Group (GIV) received only the regular rabbit diet and subject cold crud *M. albas* L. extraction of intraperitonl injection about 300mg/kg BW. At the end experiment period, rabbits were bleed and plasma sample were analyzed after collection of blood samples after an overnight fast for 12-14h and analysis perfomertion fresh heparin treated plasma. The lipid parameter Tch, TGs, LDL-c, and VLDL-c were determined by using a standard enzymatic assay (Bio-mericak-Marey-I,Eroile formlle).

Pieces of heart and liver tissues were removed for estimation of MDA by thiobarbituric acid (TBA)test as previously described by Gilbert and Rothy, (13) GSH concentration of heart and liver were measured according to Morone, etal., (14). For histopathological study, rabbit killed, heart and liver were fixed in 10% neutral buffered formalin to prepared tissue blocks that routinely embedded in paraffin, and 5-6m sections were cut. Histological sections were stained with haematoxylin and eosin (H+E), (15).

**Plant material**

The leaves of *M. albas* L. were collected freshly from Mosul city, Iraq. The leaves were air-dried at room temperature and stored in bottles until used. material was identified in department of biology in college of science, The dried leaves (250g) were powdered in electric blender, put in boiling water(1littre) for 10 min and the mixture then left to cool to room temperature. The decoction was filtered through awhtman No1filterpaper, and the resulting extract which reduced into 1/3of its volume by lophilizer, then the extraction divided into two part, first one perceptual by using aceton in order to isolation protein material from non proteinous material, while 2nd part were dried until powder formation, so cold crud aqueous extract prepared (16).

**Results**

**Chemical analysis:**

Table (1) showed that 0.5% of H$_2$O$_2$ in drinking water significantly increase in total cholesterol (Tch) triglycerides (TGS), low density lipoprotein (LDLc), very low density lipoprotein (VLDL-c) and atherogenic index, while high density lipoprotein (HDL-c) showed decreased significantly compared with untreated control rabbits. In male rabbits subjected to H$_2$O$_2$ and cold crude aqueous extract (CCAE), level of lipid profil indicated decrease of Tch, TGs, LDL-c, and VLDL-c. In these rabbits level of LDL-c increase significantly (2.77± 0.01). Table-1 with cold and boiled crud extract the picture of lipid profiles illustrated an increase of HDL-c and with concomitant decrees in Tch, LDL-c as compared with control and H$_2$O$_2$ value.

**Table (1) level of lipid profile in serum of male rabbit treated with 0.5% H$_2$O$_2$ and *M. albas* L. bold and cold crud equeous extract**

<table>
<thead>
<tr>
<th>Group</th>
<th>Tch mol/ml</th>
<th>TGS mol/ml</th>
<th>HDL-c mg/ml</th>
<th>LDL-c mol/ml</th>
<th>VLDL-c mol/ml</th>
<th>Atherogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>d2.17±0.05</td>
<td>d1.77±0.03</td>
<td>a63.0±0.01</td>
<td>b1.48±0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>a4.95±0.05</td>
<td>a1.94±0.06</td>
<td>c0.57±0.02</td>
<td>a2.77±0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H$_2$O$_2$+cold crud equeous extract</td>
<td>c2.03±0.04</td>
<td>b1.55±0.01</td>
<td>b0.58±0.02</td>
<td>d1.09±0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H$_2$O$_2$+bold crud equeous extract</td>
<td>b2.44±0.01</td>
<td>c1.60±0.01</td>
<td>d0.53±0.07</td>
<td>c1.11±0.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values are expressed as means ± S.D. of 3rabbits per group.
**Mean with same superscript in the column are significantly different from others (p<0.05).
Tissue MDA concentration:
Tissue (heart + liver) TBA reactive substances were significantly increase in rabbits after 15 days of \( \text{H}_2\text{O}_2 \) treatment as compared with control group (Table 2). The greatest decrease in MDA content was seen in heart and liver with cold crud aqueous extraction and \( \text{H}_2\text{O}_2 \) boil crud aqueous extraction also showed reduction in lipid peroxidation product as compared with \( \text{H}_2\text{O}_2 \).

Tissue GSH concentration:
Table-2 illustrated tissues (heart + liver) GSH content after 30 days of \( \text{H}_2\text{O}_2 \) treatment. There were a significant reduction in animal treated with cold and boil equeous extract of \textit{M.albas} L. treatment as compared with \( \text{H}_2\text{O}_2 \) treatment also. Treatment with extraction revealed significant increase in GSH concentration in heart and liver tissues as compared with control untreated animals (Table 2). Table-3 showed significant increase in glucose level of serum in group treated with 0.5% \( \text{H}_2\text{O}_2 \) (8.16±0.01) as compared with control. While in group treated with cold and boil crud equeous extract of mulberry leaves revealed decrease significantly as compared with \( \text{H}_2\text{O}_2 \) groups.

**Table (2) Level of MDA and GSH in heart and liver tissues of rabbit treated with 0.5% \( \text{H}_2\text{O}_2 \) and cold, boil crud equeous extract of \textit{M.albas} L. as compared with control group**

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA/mol</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>Liver</td>
</tr>
<tr>
<td>control</td>
<td>b301±19</td>
<td>b560±11</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )</td>
<td>a709±16</td>
<td>a678±19</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )+cold crud equeous extract</td>
<td>d187±13</td>
<td>d296±15</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )+boil crud equeous extract</td>
<td>c254±10</td>
<td>c350±16</td>
</tr>
</tbody>
</table>

*Values are expressed as means ± S.D. of 3rabbits per group.
**Mean with same superscript in the column are significantly different from others (p<0.05).

Table(3) level of glucose in serum of male rabbit treated with 0.5% \( \text{H}_2\text{O}_2 \), \( \text{H}_2\text{O}_2 \) and cold, boil crud equeous extract of mulberry leaves

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>6.33±0.05</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )</td>
<td>8.16±0.01</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )+cold crud equeous extract</td>
<td>5.71±0.03</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )+boil crud equeous extract</td>
<td>5.99±0.11</td>
</tr>
</tbody>
</table>

*Values are expressed as means ± S.D. of 3rabbits per group at (p<0.05).

Macroscopic lesions:
There are no macroscopic lesion observed in rabbit treated with 30 days 0.5% \( \text{H}_2\text{O}_2 \) and \( \text{H}_2\text{O}_2 \) with cold, boil crud extraction of \textit{M.albas} L.
Macroscopic lesions:

No pathological lesion were observed in the heart rabbits of control groups. Heart of groups H$_2$O$_2$ treatment revealed fatty changes, congestion of coronary arteries, infiltration of mononuclear inflammatory cells (lymphocytes) and edema between muscle fibers (Fig1).

While in groups treated with H$_2$O$_2$ and cold, boiled crud extract of M.albas L. showed sever congestion and infiltration of inflammatory cells (lymphocytes) (Fig2).

Aorta: Aortic lesions were seen in rabbits treated with H$_2$O$_2$ for 30 days characterized by presence of lipid vacuol in intimal extent to medial layers, collagen, elastin destruction spindle – shaped cells and degeneration of vascular smooth muscle cells (Fig 3,4).

In groups treated with H$_2$O$_2$ with cold and boiled crud aqueous extract, aortic section showed proliferation in vsmc and reduction in lipid vacuole in itimal in addition to, proliferation of fibroblast and collagen fibers (Fig 5).

Liver, liver of rabbit treated with H$_2$O$_2$ illustrate dilatation in sinusoid, congestion of central vein and blood, vacuolar degeneration on cytoplasm of hepatocytes, privascular and periductular of mononuclear inflammatory cells in addition to fatty change (Fig6). While in groups treated with H$_2$O$_2$ and cold and boiled crud aqueous extract revealed dilatation of sinusoid and vascular degeneration (Fig 7).

**Fig.(1) Photomicrograph of hearts rabbit treated with 0.5% H$_2$O$_2$ showed sever fatty change (a), infiltration of mononuclear inflammatory cell (b) and edema(c) H and E 900X**

**Fig.(2) Photomicrograph of hearts rabbit treated with 0.5% H$_2$O$_2$ and mulberry cold crud extract, showed infiltration of mononuclear inflammatory cell (a) and edema(b) H and E 200X**
Fig.(3) Photomicrograph of aorta treated with 0.5% \( \text{H}_2\text{O}_2 \), showed presence of lipid vacuole in intima and media (a) fragmentation of elastic membrane (b) H and E 900 X

Fig.(4) Photomicrograph of aorta rabbit treated with 0.5% \( \text{H}_2\text{O}_2 \) and boil crud extract of mulberry leaves, showed proliferation of vsmcs (a), reduction in lipid vacuole in intima (b) H and E 450 X

Fig.(5) Photomicrograph of rabbit aorta treated with 0.5% \( \text{H}_2\text{O}_2 \) and cold crud extract of mulberry leaves, showed proliferation in collagen fiber (a) and degeneration of vsmcs (b), H and E 450 X
Discussion

This study illustrated amarked elevation in level of lipid profile (Tch, TGs, LDL-c, VLDL-c). Lipid concentration in plasmas of rabbit treated with 0.5% H₂O₂ for 30 days, suggesting that these changes reflect susceptibility of animal to experimental induce hyperlipidemia and atherosis by oxidative stress that closely resemble those found in human, chicken, rat and rabbit (12, 17). This study used to test the effect of cold and boiled crude aqueous extract of white mulberry leaves as antioxidant, and antiatherogenic, the importance of mulberry leave extract as antioxidant (protective effect) take place via its content like flavonoid, which protect LDL-c from oxidation of the initial stages of lipid peroxidation by acting as free radical scavenger (18,19). Quercetin and flavonoid were shown be bind to the surface of LDL particles via the formation of an ether bond (20). Mulberry extraction, both cold and boil crude extract a significantly amelioration effects on lipid peroxidation and antioxidant status can decrease in level of Tch, TGs, LDL-c, VLDL-c and atherogenic index (Table 1) in addition to, decrease concentration of MDA heart and liver tissues associated with increase level of HDL-c in serum and GSH of heart and liver tissues. Our study suggest that flavonoid and ascorbic acid, important continent leave of mulberry and play important role in prevention endothelial cell-mediated LDL-c lipid peroxidation (21). However mulberry leaves extract reduced the tendency of LDL-c oxidize and enhenc antioxidant enzyme activity of cells (22). This study revealed that no protection effect of this dose of mulberry leave equeous extract was observed at the histopathological levels. Thus, the protective effect of mulberry equeous extract leaves agents H₂O₂
induced oxidative stress and atherosclerosis may be due to changes in the antioxidant enzyme chain breaking antioxidant activity and have antiproliferative properties and its ability to inhibit redox-sensitive signal transduction pathway related to cell growth on vsmCs (23). In this study, the mulberry treatment inhibited the increase in lipid peroxidation of rabbit as compared with the H2O2 treated group. Plasma glucose levels were also reduced in the mulberry equeous extract rabbits. It was suggested that this effect is related to polyhydroxylated alkaloids, including 1-deoxynojirimycin, α-glucosidase, fugomine, which potentials glucose induced insulin release and to the increase in tissue uptake of glucose by the leaves (24,25, 26).

In conclusion, our study found that treated with mulberry leaves equeous extract (cold and boilid) was inhibited the effect of H2O2 induced changes except the histopathological one, therefor, the use of mulberry as antiatherotic effect of rabbit illustrated less histopathological effect on aortic lesion via less reduction in lipid vacuoles and induced proliferation of vsmCs in media toward intima.

References