Hepatoprotective and Antioxidant Activity of Wheat Germ Oil Against Nicotine Induced Oxidative Stress

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Abstract: Rational: Smoking kills more than one billion current smokers worldwide prematurely due to various tobacco-related diseases. Smoking contributes to multiple well-documented adverse health effects, including heart disease, pulmonary disease and lung and other cancers in both industrialized and developing countries. Tobacco smoking is a leading cause of preventable disease and death. If the current trend remains unchanged, the annual number of deaths due to tobacco will reach more than 10 millions by 2025. People who quit smoking can, for the most part, reverse these risks. Oxidative stress is believed to be involved in the pathophysiology of a number of chronic diseases including liver diseases, atherosclerosis, diabetes and cataracts. Objective: This study was aimed to assess the protective effect of wheat germ oil the role of wheat germ as antioxidant and its value in protection of liver cells against the in vivo modulation of nicotine induced oxidative stress using rat as a model. Setting and Design: Thirty six male albino rats (Sprague Dawley), were classified into three groups (12 animals per group). Gr. I: Served as control group, where animals were received 0.1 ml corn oil for successive thirty days. Gr. II: Served as nicotine group, where rats received intraperitoneal (ip) injection of 0.5 mg nicotine base /Kg body weight. Gr. III: Served as wheat germ oil treated group pre-injected with nicotine, where rats received injection of 0.5 mg nicotine base /Kg body weight (ip) followed by daily administration of wheat germ oil (54mg/kg body weight dissolved in 0.1 ml corn oil), orally for successive thirty days. At the end study terminated, animals sacrificed and blood was drawn from the heart and sera were kept in fridge until time of biochemical analysis as well as liver tissues were collected for both biochemical assessment and histopathological evaluation. The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS 16 software package. Results revealed that in The results showed that food intake and body weight gain of rats injected with nicotine were significantly lower than those of control rats. Serum liver enzyme glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) activities and total bilirubin (Bt) were significantly increased due to injection of nicotine (ip). The homogenate livers were decreased significantly in superoxide dismutase (SOD), glutathione peroxidase (GSH-Px and nitric oxide (NO) activity in addition to non-protein sulfhydryl groups and vitamin E when rats injected with by nicotine (ip). On the other hand, a significant increase in lipid peroxidation products was shown by measuring malondialdehyde (MDA). A significant improvement was recorded in rats administrated wheat germ oil (WGO) parallel with rats injected intraperitoneal with nicotine for thirty successive days. The histopathological examination of the liver tissues of animals injected with nicotine showed different lesions but administration with wheat germ oil (WGO) caused an improvement in liver as compared with nicotine group. In Conclusion: Oral administrations of wheat germ oil are effective in reducing the toxic effect of nicotine and are also effective in oxidative stress damage produced by nicotine. So wheat germ oil is recommended to be given to individuals who are exposed to environments polluted with nicotine.

Key words: Nicotine • Smoking • Liver • Antioxidant • Free Radicals • Wheat Germ Oil

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INTRODUCTION

Plant origin food ingredients are the main source of very potent antioxidants. Wheat germ oil the natural antioxidants are very potent and when implemented into cell membranes are able to scavenge a large number of free radicals. Cigarette smoking, tobacco chewing and nicotine replacement therapies are the main sources of human exposure to nicotine [1]. Nicotine is the principal alkaloid in tobacco plant \textit{(Nicotina tabacum)}, that has a variety of activities in the body and central nervous system (CNS) acting largely as a stimulant via activation of nicotinic receptors. Cigarettes typically have 10-25 mg of nicotine each and peak plasma nicotine levels are higher with cigarettes than with replacement products [2]. The liver metabolizes 80 - 90 % of nicotine, small amount metabolized in lung and kidneys [3]. Cytochromes P-450 (CYPs) are the primary enzymes involved in hepatic phase I metabolic reactions, nicotine is inactivated to cotinine primarily by hepatic CYP2A6 [4]. Activation of CYP-dependent mono-oxygenases by nicotine give rise to metabolic formation of electrophilic metabolites which bind to nucleophilic constituents of the cell and induce oxidative stress through out the generation of reactive oxygen species (ROS) [1, 5].

When an imbalance occurs between oxidants and defense system, oxidative stress occur. Cells have several ways to alleviate the effects of oxidative stress. They can either repair the damage or directly reduce the pro-oxidative state via enzymatic and non-enzymatic antioxidants [6]. Wheat germ oil, which makes up only 7-12% of the seed, is an excellent source of natural vitamin E and tocopherols, the richest known source in nature [7]. Wheat germ oil is also rich in unsaturated fatty acids, mainly oleic, linoleic and \( \alpha \)-linoleic acids [8] and in functional phytochemicals, mainly flavonoids, sterols, octacosanols and glutathione [9]. The aim of the present study is to elucidate the role of wheat germ as antioxidant and its value in protection of liver cells against the \textit{in vivo} modulation of nicotine induced oxidative stress using rat as a model.

MATERIALS AND METHODS

Materials: The present study was carried using male albino rats, weighing 120-150g obtained from animal house unit of El-Nasr pharmaceutical chemical Co. Abou-Zaabal Cairo. Animals were acclimatized in our animal house, in Pharmacology Dep. Faculty of Pharmacy Al-Azhar University for one week before start of the experiment. They were apparently normal, healthy animals. Animals were fed on standard rodent pellet diet with water. Wheat germ oil was supplied as soft gel and was provided by MEPACO company. Wheat germ oil (300 mg/5ml) was suspended in corn oil and administered to animals by gavage at doses 54 mg/kg body weight. The doses were selected on the basis of the reports from previous studies [10].

Experimental Design: Animals were classified into three groups (12 animals per group). Gr.I: Served as control group, where animals were received 0.1 ml corn oil for successive thirty days. Gr. II: Served as nicotine treated group, where rats received injection of 0.5 mg nicotine base /Kg body weight i.p (Intraperitoneal) [11]. Nicotine was freshly prepared and injected once per day. 0.5 mg nicotine/Kg. Body weight was tested in rats to be tolerable dose with less mortality rate [12]. Gr.III: Served as wheat germ oil treated group pre-injected with nicotine, where rats received injection of 0.5 mg nicotine base /Kg body weight (i.p) followed by daily administration of wheat germ oil (54mg/kg body weight dissolved in 0.1 ml corn oil), orally for successive thirty days.

Biological Evaluation: Feed intake, body weight gain (BWG %), feed efficiency ratio (FER) and organs weight relative to body weight % were calculated [13]. During the experimental period, the body weight was recorded every week. Clear separated serum were subjected to the following biochemical analysis. Liver, kidney and heart were removed from each rat, carefully washed with saline solution, dried with filter paper and weighted [14].

Biochemical Analysis: At the end of the experimental period, all animals were sacrificed 24 hrs after the last injection, sera were separated from collected blood samples by centrifugation and used freshly for determination of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase activities were determined [15]. Total bilirubin [16]. A midline abdominal incision was performed in each animal and livers were dissected, washed with saline, sliced and homogenized in ice cold 50mM sodium phosphate buffer (pH 7) containing 0.1 EDTA to give 10% (w/v) crude homogenate. The homogenate was centrifuged at 2000rpm for 15 minutes at 4°C and the supernatant was used for determination of SOD activity.
non-protein sulfhydryl groups were determined using Ellman's reagent [18], lipid peroxidation products were assayed by malondialdehyde (MDA) [19] and glutathione peroxidase (GSH-Px) by HPLC [20], vitamin E was measured [21], nitric oxide (NO) was measured [22] and protein concentration was measured [23], using bovine serum albumin as standard.

Histopathological Examination of Liver: Liver were dissected out and fixed instantaneously in 10% formal saline for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax at melting point 55-60 °C. Sections of 6mm thickness were prepared and stained with haematoxylin and eosin [24].

Statistical Analysis: Data are presented in tables as means ± standard deviation (S.D.). Values were statistically analyzed by one-way analysis of variance (ANOVA) [25] by using SPSS 16 software package. The P values <0.05 were considered significant.

RESULTS AND DISCUSSION

Biological Evaluation: Table (1) showed that feed intake, body weight gain (BWG%) and ratio (FER) of rats injected with nicotine were significantly lower than these of control rats. The highest significant increased (p<0.05) of feed intake, (BWG%) and (FER) was in group administrated with wheat germ oil parallel with nicotine injection (WGO/NICO) (10.21±0.6 g/day, 12.52±0.7% and 0.82±0.6, respectively ) as compared with nicotine group and normal rats group.

The net body weight gain of the animals intoxicated with nicotine was markedly less as compared to the normal controls, suggesting that the poor body weight gain may be due to the overall increased degeneration of lipids and proteins as a result of the direct effects of nicotine. Many animal studies reported that exposure to organic solvent reduced feed intake and body weight gain in mice and in rats, which this effect may be due to the loss of appetite [26, 27].

Biochemical Analysis: Table (2): Effect of nicotine and wheat germ oil on liver functions:

The results of the present study showed that nicotine administration was accompanied by significant increase in liver function tests as SGPT, SGOT (U/L) and Bt (mg/dL). As shown in the table, mean values of nicotine group were significantly higher (p<0.05) when compared with normal rats group. The administrated with wheat germ oil parallel with nicotine injection (WGO/NICO) showed significant decrease (p<0.05) of liver enzyme and total bilirubin (50.6±7.0, 37.0±4.1 and 1.6±0.41, respectively ) as compared with nicotine group.

Table 1: Body weight gain (BWG%), feed efficiency ratio(FER) and feed intake and organs weight/ body weight % for hepatotoxicity rats and wheat germ oil/nicotine group (n=12 rats)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Feed intake (g/day)</th>
<th>BWG%</th>
<th>FER</th>
<th>Liver</th>
<th>kidney</th>
<th>heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>11.42±0.3</td>
<td>25.88±0.4</td>
<td>0.44±0.5</td>
<td>3.0± 0.12</td>
<td>0.94±0.03</td>
<td>0.44±0.03</td>
</tr>
<tr>
<td>Nicotine group</td>
<td>5.33±0.7</td>
<td>5.65±0.6</td>
<td>0.49±0.2</td>
<td>4.6±0.37</td>
<td>0.72±0.05</td>
<td>0.32±0.04</td>
</tr>
<tr>
<td>WGO/NICO</td>
<td>10.21±0.6</td>
<td>12.52±0.7</td>
<td>0.82±0.6</td>
<td>3.7±0.28</td>
<td>0.89±0.02</td>
<td>0.40 ±0.02</td>
</tr>
</tbody>
</table>

Mean: SD values, means in the column with different letters are significantly different (p ≤ 0.05).
Table 2: GPT, GOT (U/L) and total bilirubin (mg/dL) in all studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGPT U/L</th>
<th>SGOT U/L</th>
<th>Bt mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats group</td>
<td>15.17±3.2</td>
<td>11.5±1.6</td>
<td>0.17±0.05</td>
</tr>
<tr>
<td>Nicotine group</td>
<td>84.83±7.9</td>
<td>68.7±4.6</td>
<td>3.9±0.26</td>
</tr>
<tr>
<td>WGO/NICO</td>
<td>50.6±7.0</td>
<td>37.0±4.1</td>
<td>1.6±0.41</td>
</tr>
</tbody>
</table>

Mean: SD values, means in the column with different letters are significantly different (p < 0.05).

Table 3: Oxidative stress markers for hepatotoxicity rats and wheat germ oil/nicotine group (n=12 rats)

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD U/mg protein</th>
<th>GSH-Px U/g protein</th>
<th>MDA nmol/g protein</th>
<th>NO µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats group</td>
<td>47.4±6.3  c</td>
<td>65.3±5.5  a</td>
<td>42.9±3.7  a</td>
<td>6.0±0.46  a</td>
</tr>
<tr>
<td>Nicotine group</td>
<td>12.8±2.3  a</td>
<td>22.3±4.8  c</td>
<td>70.3±3.8  c</td>
<td>14.9±0.92 c</td>
</tr>
<tr>
<td>WGO/NICO</td>
<td>25.0±3.9  b</td>
<td>38.8±3.5  b</td>
<td>53.0±2.8  b</td>
<td>8.8±0.81 b</td>
</tr>
</tbody>
</table>

Mean: SD values, means in the column with different letters are significantly different (p < 0.05)

Liver, the major metabolic site, is highly susceptible to the oxidative events associated with nicotine toxicity. When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream. Elevation of SGPT and SGOT indicates liver damage [34]. In our study, nicotine administration was accompanied by significant increase in liver function tests as SGPT, SGOT (U/L) and Bt (mg/dL), such increase was regarded as alterations in cell membrane, resulting in increased membrane permeability and leakage of liver enzymes from hepatocytes to circulation.

Previous data agreement with Rebekka Kubisch et al. [35] who studied the cytotoxicity or cellular effects induced by inorganic ions (Ni2+ and Cu2+) can be detected with the metabolic parameters acidification and respiration down like nicotine and acetaminophen are rather high, in the range of 0.1 mg/L and 100 mg/L. The results support the paradigm change from single substance detection to the monitoring of overall toxicity. Liver damage by increasing the activities of serum enzymes SGPT, SGOT, bilirubin [36].

Also, the results of the present study indicate that wheat germ oil (WGO) significantly reduce the toxic effect of nicotine by altered the hepatic enzyme activities and thus can be considered a potential hepatoprotective agent. Our data agreement with Abdel Fattah, Fahim and El-Fatih [37], who found that, the rats that received combined treatment with wheat germ oil and panax ginseng supplement showed significantly less severe damage and remarkable improvement in all of the measured parameters when compared to irradiated rats and concluded that, wheat germ oil and panax ginseng supplement might be a useful candidate against radiation-induced oxidative stress and metabolic disorders without any toxicity.

Table (3) showed effect of nicotine and wheat germ oil on endogenous antioxidant status: Serum oxidative stress markers as SOD (U/mg protein), GSH-Px (U/g protein), MDA (nmol/g protein) and NO (µmol/L) in homogenate livers of all groups. As shown in this table, mean values MDA for nicotine rats group was significantly increased (p < 0.05) when compared with normal rats group, while the administration of wheat germ oil parallel with nicotine injection (WGO/NICO) showed significant decrease (p <0.05). On the other hand, SOD, GSH-Px and NO of nicotine rats group recorded significant decrease (p <0.05) when compared to normal rats group. These values are improved by administration rats with wheat germ oil parallel with nicotine injection (WGO/NICO) and recorded significant increase when compared to nicotine group.

These results are agreement with Anshu Jain and Flora [38] who indicate that, brain lipid peroxidation increased at all three doses of nicotine in young as well as old rats as compared to their respective normal control. Tissue homogenates prepared from the tissue specimens were analyzed for malondialdehyde (MDA) levels and catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities. It was determined that coumaphos led to adverse alterations in the majority of the oxidative stress markers investigated [39]. The administration of wheat germ oil alleviated the coumaphos-induced adverse effects detected in the tissues examined. Zeinab et al. [40], showed that rats fed the diet supplemented with carrot or wheat germ oil along with benzene had a reduction in the level of MDA (p <0.01), whilst their GSH concentration, SOD, GSH-Px CAT activity and vit. E levels were improved. Also, the significant decrease in hepatic superoxide dismutase (SOD) levels reflects those of hepatic injury, indicating the possibility of a nicotine-induced down regulation of
SOD enzyme production [41]. The liver is the main detoxifying organ in the body and as such it possesses a high metabolic rate and it is subjected to many insults potentially causative of oxidative stress [42]. Consequently, a correct status of the hepatic antioxidant defense system is of major importance for the maintenance of health. Highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce malondialdehyde, a lipid peroxidation product [43]. This is agreement with our results since nicotine have been reported to induce oxidative stress, as shown by enhanced MDA production.

Oxidative stress caused by various agents (toxins, metals, dioxin and pesticides) is considered as an imminent threat for many organisms since it can lead to death. However, the imbalance between production of oxygen free radicals (OFRs) and antioxidant defenses in the body is called oxidative stress which has important health implications reported by Ranjbar et al.[44]. If there are too many OFRs or too few antioxidants for protection, a condition of oxidative stress develops, which may cause chronic damage [45].

The liver is the most sensitive organ to preoxidative damage because it is rich in oxidizable substances. The increment of the oxidative stress on the cells of the liver and the consequent decrease in the antioxidant ability of the cells result in the occurrence of aggressive cellular damage to the liver cells with destruction of their membranes and the release of the enzymes into the blood stream. The more severe the liver damage the higher the release of the liver enzymes [46].

Biological systems have evolved with endogenous defense mechanisms to help protect against free radical induced cell damage. Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) are antioxidant enzymes, which metabolize toxic oxidative intermediates. They require micronutrient as cofactors such as selenium, iron, copper, zinc and manganese for optimum catalytic activity and effective antioxidant defense mechanisms [47, 48].

Table (4) showed mean values of antioxidants as NPSH group (nmol/mg protein) and Vit. E (µg/dL) in homogenate livers. As shown in this table, the results showed that, nicotine group was significantly lower ($p<0.05$) when compared with normal rats group. The administrated with wheat germ oil parallel with nicotine injection (WGO/NICO) showed significant increase ($p<0.05$) 24.3±4.9 and 500.3±27.9 respectively, when compared to nicotine rats group.

Glutathione directly protects membrane proteins and lipids and preserves their stability. Decreased levels of glutathione lead to a decrease in -SH groups [49] and can result in the oxidation of membrane -SH groups and loss of membrane stability [50]. Antioxidants have been shown to inhibit free radical formation [6]. Natural antioxidants such as vitamin E, A, β-carotene and vitamin C play a key role in promoting defense mechanism against oxidative stress [51, 52]. Wheat germ is also rich in unsaturated fatty acids, mainly oleic and γ-linoleic acids [8] and in functional phytochemicals, mainly flavonoids, sterols, octacosanols and glutathione [53]. Wheat germ oil is unique among dietary supplements, it is highly rich in the most biologically active forms of naturally occurring vitamin E and mixed tocopherols [54].

Vitamin E acts as an inhibitor of oxidative processes in body tissues, it protects the unsaturated fat in the body from oxidation. It has been reported that oral administration of wheat germ oil efficiently saturates the body of rats with vitamin E and inhibits oxidation [55]. These data were in agreement with many other reports [56, 57]. Thus vitamin E can be given as a nutritional supplement to reduce oxidative stress. These active substances play an important role in increasing liver tissue levels of non-protein SH group and vitamin E and promoting defense mechanism against oxidative stress.

**Histopathological Examinations of Liver:**

Microscopically, liver of rat from control normal group revealed the normal histological structure of hepatic lobule (Fig1.). However, liver of nicotine-intoxicated rat without treatment showed kupffer cells activation, cytoplasmic vacuolization of hepatocytes (Fig1b and Fig1c) dissociation of hepatocytes and portal infiltration with leucocytes. Kupffer cells activation was the only histopathological change observed in liver of hepato-intoxicated rat administered with WGO/NICO (Fig1d and Fig1e). With respect to the hepatic histo-architecture of the nicotine injected rats there was an increased vacuolization of hepatocytes and focal necrosis in comparison to untreated normal controls. The congestion

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**Table 4: Antioxidants for hepatotoxicity rats and wheat germ oil/nicotine group (n=12 rats)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>NPSH group nmol/mg protein</th>
<th>Vit. E µg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats group</td>
<td>41.2±3.4 c</td>
<td>700±42.0 c</td>
</tr>
<tr>
<td>Nicotine group</td>
<td>12.8±2.3 a</td>
<td>300±43.5 7 a</td>
</tr>
<tr>
<td>WGO/NICO</td>
<td>24.3±4.9 b</td>
<td>500.3±27.9 b</td>
</tr>
</tbody>
</table>

Mean± SD values, means in the column with different letters are significantly different ($p<0.05$).
of the portal area, inflammatory infiltration increased in these animals. These observations indicated marked changes in the overall histo-architecture of liver in response to nicotine, which could be due to its toxic effects. Primarily by the generation of reactive oxygen species causing damage to the various membranous components of the cell. The necrotic conditions observed in liver of the nicotine injected rats are in corroboration with the observed biochemical changes, wherein an increased level of lipid peroxidation was noticed. The administration of wheat germ oil (WGO) is recommended as a concomitant supplement to the routine therapy for the protection against severe tissue damage induced by nicotine.

These findings are agreement with Yuen et al. [58], who illustrated that, histology demonstrated a significant hepatotoxic effect in the group receiving 108 mumol/l of nicotine when compared with the control group in the form of fatty change, focal or confluent necrosis and dark-cell change. The lymphocytic infiltration observed in this study following nicotine injected indicates signs of irritability, inflammation and hypersensitivity to the toxicant used. In the present study Kupffer cells increased in number. This can be explained by the fact that these cells are supposed to be hepatic macrophages which act as phagocytic cells species causing damage to the various membranous components of the cell. The necrotic conditions observed in liver of the nicotine injected rats are in corroboration with the observed biochemical changes, wherein an increased level of lipid peroxidation was noticed. The administration of wheat germ oil (WGO) is recommended as a concomitant supplement to the routine therapy for the protection against severe tissue damage induced by nicotine.

From the afore mentioned results, it can be concluded that nicotine administration significantly toxicates hepatocytes by increasing lipid peroxidation and decreasing the enzymatic and non-enzymatic antioxidants defense status as well as the increased incidence of focal and confluent necrosis.

Evidence are emerging that wheat germ oil supplementation can have beneficial effects on liver functions as well as oxidative stress markers. So, the present study highlights the protective role of some foodstuffs, such as wheat germ oil (WGO), in reducing the degree of oxidative stress induced by nicotine.
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