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Protective Effect of Luteolin and Quercetin Supplements on Nicotine Induced Oxidative Stress and DNA Damage in Young Rats

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Abstract

SECOND-HAND SMOKING poses a serious threat to children`s health, leading to increased risks of illness and death. We targeted to examine how luteolin (Lu) and/or quercetin (Qu) supplements could protect against oxidation and DNA damage are caused by nicotine in young rats. The Lu effect was compared with Qu. Fifty rats were divided to five groups, as follows: (1) control group without treatment; (2) only nicotine (Ni) (0.75 mg/kg); (3) Qu supplement (50 mg/kg/day) with nicotine; (4) luteolin supplement (50 mg/kg/day) with nicotine; and (5) combination of Qu and Lu (50 mg/kg) for each along with Ni. After 8 weeks, serum, liver, and lung samples were collected. Rats` body weight parameters showed considerable decreases in the Ni group compared to other groups. The liver function enzymes, and malondialdehyde (MDA) value were considerably increased in Ni rats when compared to the control group. The Ni rats showed a notable reduction in endogenous antioxidant enzymes. The Lu and combined groups showed considerable enhancement in all mentioned parameters compared to the Ni group. At the cellular level, the extent of DNA damage was significantly increased in Ni-injected rats, which showed no fragmentation in the Lu+Qu combined administered group. The prophylactic impact of Lu versus nicotine oxidation was similar to Qu. Data indicated that Lu functions by enhancing the antioxidant defense mechanism, modulating lipid peroxidation levels, and ultimately safeguarding DNA in serum and tissues. Combining Lu and Qu led to a synergetic effect, leveraging their respective antioxidant abilities to combat the nicotine oxidative stress.

Keywords: Nicotine, Oxidative stress, Luteolin, Quercetin, DNA damage.

Introduction

Human frequently encounter nicotine, a prominent substance in daily life. Tobacco use stands as a major contributor to global health risks, diseases, and fatalities. If the current tobacco use pattern persists, we anticipate approximately 8 million deaths annually by 2030. Second-hand smoke (SHS) exposure impacts an estimated 1.3 million non-smokers [1]. Currently, there are almost 1.1 billion smokers worldwide [2]. According to a systematic review, the highest rates of smoking among university students were observed in Egyptian 46.7%, Kuwaiti 46% and Saudi Arabian 42.3% (n=45,306) from all Arab countries [3]. Recent research revealed that the smoking prevalence among Egyptian adolescents in Greater Cairo was 12.8% [4]. Saudi Arabia ranks eighth globally in terms of tobacco use [5], while Egypt ranks ninth in terms of raw tobacco imports. Annually, smoking in Egypt leads to the deaths of 170,000 individuals. Despite increased measures to discourage smoking, the rate of smoking has increased rapidly in the recent decade [6].

Nicotine, the main active alkaloid found in tobacco, plays a key role in sustaining smoking habits. It`s distributed very quickly. The blood-brain barrier absorbs nicotine in almost 7 seconds, and its half-life is approximately 2 h [7]. Nicotine enters the body in different ways: by inhalation during smoking, and by mouth through the tobacco products, which are quickly absorbed into the circulation [7]. The liver metabolizes nicotine into its primary compound, cotinine, which in turn generates free radicals in the tissues. The development of

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radicals with glutathione reduction in tissues causes oxidative damage [8]. It was proven that nicotine administration (2 mg/kg) generates oxidative damage in both the liver and kidney. One cigarette, whether smoked positively or negatively, produces 11 mg of nicotine, with an average absorption of 1 mg [7]. Nicotine also causes genotoxic effects in rat liver, in human target cells and in blood peripheral erythrocytes [9]. As a result, individuals who do not smoke, especially children, are at an increased risk of developing heart disease by 25-30% and lung cancer by 20-30% due to exposure to SHS at home or in the workplace [10]. Every year, exposure to SHS puts between 150,000 and 300,000 children under the age of 18 months at risk for respiratory tract infections, severe asthma, and even death. In addition, over the past five decades, greater than 100,000 infants have succumbed to sudden infant death syndrome (SIDS) as a result of their guardians smoking [10, 11]. An increasing number of studies have demonstrated that there is a detrimental effect of cigarette smoking or nicotine on oxidative stress and DNA damage. [9, 10,12].

Flavonoids are plant biologically active polyphenol compounds, which are classified as a group with the ability to shield cells from harmful influences [13]. Flavonoids are classified as flavonols, flavones, isoflavones, and anthocyanidins. Among natural flavonoids, luteolin is one of flavones and quercetin is one of flavonols that are widely distributed in plant-derived foods and commonly used as dietary supplements. In nature, luteolin is rich in herbs and vegetables such as cabbage, lettuce, thyme, parsley, and rosemary, while quercetin is rich in green vegetables, berries, legumes, green tea, citrus fruits, and red grape [15, 16]. Luteolin possesses many beneficial properties including antioxidant, anti-inflammatory, and anti-proliferative [15]. Quercetin has a wide range of implications as a powerful antioxidant, leads to the possible preventive and therapeutic advantages of it in specific disease conditions [16]. Although quercetin has been the most explored flavonoid in the last decade [17], current studies claim that luteolin could be a more promising flavone as an antioxidant but needs additional investigation to confirm [18]. Despite all mentioned health benefits of Lu, its utility is limited by factors such as low solubility, inadequate absorption, and rapid metabolism [19]. To address these constraints, the combination treatment of luteolin with other well explored flavonoids, such as quercetin, needs additional investigation to establish a hopeful remedy against common oxidants such as nicotine. Therefore, this study objected to examine the adverse effects of nicotine and protective (additive or synergistic) impacts of luteolin and quercetin supplements in the blood and tissue of young rats.

Material and Methods

Chemicals

Nicotine hydrogen tartrate was obtained from Sigma Aldrich, in South Africa. Phosphate Buffered Saline (PBS) was obtained from LonzaWalkerville in USA, while and Dimethyl Sulfoxide (DMSO) was sourced from Biomatik in Ontario, Canada. The quercetin capsules (500 mg) were procured from General Nutrition Centers (GNC), Inc., USA. Luteolin capsules (50 mg) supplement was from Supersmart, Smart Nutrition S.A, Luxembourg.

Experimental animals

Young male Wister rats were obtained from the animal facility of King Fahd Medical Research Center at King AbdulAziz University, Jeddah, Saudi Arabia. Throughout the experiment, rats were housed in standard plastic cages environmentally maintained at temperature (25°C ± 2°C), and clamminess (55% ± 5%), with a daily cycle of 12-hours of light followed by 12 hours of darkness for at least one week to adapt. The animals had unrestricted access to food and water throughout the entire study period.

Experimental protocol:

Fifty young male Wistar albino rats (70-80g; 4 weeks old) were randomly assigned to five groups for an 8-weeks treatment regimen as outlined: (1) the control group (Cont) received intraperitoneal (ip) injections of normal saline (0.1 mL/ kg/day) coupled with 1% DMSO dissolved in PBS solution as a vehicle. Groups from two to five were injected with nicotine (0.75 mg/kg/d) all over the experimental time. Group two kept on nicotine only (Ni) as the positive group, coupled with 1% DMSO dissolved in PBS solution as a vehicle. While groups three and four were administered with quercetin (Ni+Qu) and Luteolin (Ni+Lu), respectively with concentration (50 mg/kg/d) for each by intragastric tube. The last group five received combination of quercetin and luteolin at doses of 50 mg/kg/day each along with Ni (Ni+Lu+Qu). The used doses were applied according to previous literature data [20, 21, 22] and were related to human daily dietary intake of quercetin-rich foods and daily nicotine intake in individuals who smoke 10-20 cigarettes daily [23]. The nicotine in normal saline was prepared freshly for dosage and ip injected per day at 11.00 a.m. The Lu and Qu supplement doses were prepared once a week in 1% DMSO dissolved in PBS solution, and administered using an intragastric tube per day for 8 consecutive weeks at 10.00 a.m. [20]. During the experiment, rats were handled in accordance with international ethical guidelines for laboratory animal care. All experimental protocols were approved by the Research Ethics Committee of King Abdulaziz University, Saudi Arabia, under the project number of (1-17-04-009-0007).

The rat weight was recorded weekly throughout the study period, which was used to calculate the doses of Ni, Lu and Qu. The liver, lung and brain coefficients, reflecting the ratio of the organs` wet weight (measured in milligrams) to the final body weight of the rat, were computed as follows:

Relative organ weight (%) = (wet organ wt (g)/Final body wt (g) × 100)

Body weight gain = (Final body wt – Initial body wt) / Initial body wt

Preparation of serum and tissue samples

At the end of the experimental period, the rat weights were recorded and fasted overnight for 13-16 hours. The rat was anesthetized, blood samples were withdrawn from the veins near the inner corner of the eye, as retro-orbital plexuses, at time between 8-10 a.m. Blood tubes were centrifuged at 3000 rpm for 15-20 min at 4°C to detach serum, which was kept at -80 °C for later biochemical analyses.

Immediately after serum collection, and decapitation of the rats, the liver and lungs were quickly resected, washed, dried, and weighted. Hepatic and lungs tissues were used to prepare 10% (w/v) organ homogenate in a ratio of 0.1 g tissue for 5 ml cold assay buffer using a homogenizer. The homogenized tissues were centrifuged at 10,000 × g for 15 min at 4°C. The upper fluid was collected and stored at -80°C in order to analyse DNA damage.

Biochemical analyses:

Liver function enzymes activity in serum was made using an automated clinical chemistry analyzer (ELITech Group Co., France) to analyse and determine the Alanine aminotransferase (ALT) (EC 2.6.1.2), aspartate transaminase (AST) (EC 2.6.1.1) and Lactate dehydrogenase (LDH) (EC 1.1.1.27) [24, 25].

The endogenous antioxidant enzymes activity was determined including Superoxide dismutase (SOD) (EC 1.15.1.1), Catalase (CAT) (EC 1.11.1.6), and Glutathione peroxidase (GPx) (EC 1.11.1.9), in addition to non-enzymatic antioxidant Reduced glutathione (GSH) [26, 27]. Quantification of lipid peroxidation as malondialdehyde (MDA) was assessed to display the role of oxidative injury in pathophysiological disorders [28].

Rat`s liver and lung tissues, along with serum samples were utilized to quantitatively assess the extent of DNA fragmentation using diphenylamine reaction procedure. The method based on releasing of DNA out of cell using lysis buffer. The DNA`s deoxyribose, in acidic condition, forms β hydroxyl leavulinic aldehyde compound, which is reacts with diphenylamine to give a bluish green color that has an absorption at 595 nm [29]. Additionally, apoptotic DNA fragmentation was qualitatively examined by identifying the ladder pattern in nuclear DNA [30]. In this method the taken weight from liver and lung tissue homogenates were 50 µg, while taken volume was 500 µl from serum samples to enrolled in DNA extraction steps as described by Lu and colleagues [30]. The concentration of the agarose in this method was 2%, which is usually used in most of DNA fragmentation methods.

Statistical analysis

All statistical analyses were conducted using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). The results are presented as mean ±SEM and were assessed using a one-way analysis of variance (ANOVA) test. Statistical significance was established at $p \leq 0.05$ and $p \leq 0.01$ for all comparisons.

Results

Body and organ weight parameters:

The effect of nicotine, Lu and/or Qu supplements on body weight parameters is shown in Table (1). The findings displayed significant reductions ($p \leq 0.01$) in the final body weight and weight gain percentages of the Ni group compared to the normal (Cont) and supplement groups. Otherwise, there were considerable differences ($p \leq 0.05$) among the Qu and Lu groups in final body weight and weight gain, with no obvious difference in weight gain percentage. There was a significant decreasing effect of nicotine on body weight parameters, which enhanced with the administration of quercetin and luteolin supplements.

The effect of nicotine, Lu and/or Qu supplements on liver, lung and brain weight and their relative weight ratio of young rat groups are shown in figure (1). Results indicated a substantial decrease with high significance ($p \leq 0.01$) on all organs weight and their relative weight ratio in Ni group compared to Cont group. While Lu and combined supplements group showed notable enhancements ($p \leq 0.01$) on all organ weights and relative ratios compared to nicotinic group. When comparing supplemented groups with each other, significant differences ($p \leq 0.05$) between Qu and Lu groups were detected on liver and brain weight and relative ratios, with no obvious differences on lungs weight. The most affected organ by a nicotinic injection was the brain followed by liver and then lungs. Among all supplemented groups, the Lu group and combined group showed the most enhancement against the harmful effect of nicotine on all studied body organs of young rats.

Liver-function enzymes

Table 2 displays the activity of serum liver enzymes (ALT, AST and LDH) in both the nicotine and supplement groups of young rat. The Ni-injected rats showed a high significant increase ($p \leq 0.001$) on all liver enzymes activity compared to the Cont and

supplemented groups. Interestingly, the quercetin supplementation showed the most enhancement on all liver enzymes among three supplemented groups, followed by combined group, while luteolin group didn`t show the same effect. But no obvious differences were detected between Lu and combined groups for all three liver function enzymes. There were significant negative effects of nicotine on serum liver function enzymes in young rats.

Endogenous antioxidant status:

To evaluate the oxidative stress that is induced by nicotine injection, Figure (2) represents the effect of Ni and Qu and/or Lu administration on endogenous antioxidant markers (CAT, GPx, SOD and GSH), and lipid peroxidation markers (MDA) as well. The results revealed considerable reduction ($p \leq 0.01$) in all evaluated antioxidant markers caused by Ni injection comparable to other groups. These reductions were more pronounced in GPx and CAT enzymes. While showed remarkable increasing ($p \leq 0.01$) on MDA level in serum of Ni rats compared to Cont and supplemented rats. All supplemented groups showed a significant increment on antioxidant markers, and significant reduction on MDA when compared to Ni group. Among all supplemented groups, Lu and combined (Ni+Qu+L) groups showed the best recovery to normal levels for CAT, GPx and GSH antioxidant markers and for MDA as well compared to Qu group (Figure 2).

DNA fragmentation:

Figure 3 summarized the rate of DNA fragmentation in serum, lungs and liver tissues, which was analyzed by the diphenylamine reaction procedure (left side histograms) and detection with agarose gel electrophoresis using ladder (right side). The results revealed that rate of DNA fragmentation was increased significantly ($p \leq 0.01$) in rats exposed to (Ni) compared to Cont and supplemented groups on serum, liver and lungs tissue. However, all supplemented groups showed high significant ($p \leq 0.01$) lowering in DNA fragmentation rates compared to nicotine (Ni) group for all studied samples. Furthermore, the combined supplements (Ni+Qu+Lu) group showed the lowest rates of DNA fragmentation, which were relatively similar to Cont group for serum samples. On the other hand, the agarose gel electrophoresis of Ni group showed fragmented DNA bands more than Cont group for all samples. While, the Qu and Lu groups showed lower fragmented DNA bands compared to (Ni) group for all samples. Moreover, the combined (Ni+Qu+Lu) group showed no fragmented DNA bands, which were relatively similar to (Cont) group for all samples.

Discussion

Nicotine is addicted by millions of smokers worldwide. It has many negative effects on many organs, particularly on liver and lung. We used nicotine because it's the major component of tobacco, which may be enter the body by positive or negative smoking and affect directly to children. The widespread adverse health effects of cigarette smoke worldwide are substantial, tobacco use continues to rise on a global scale [23].

In the beginning, we thoroughly examined many supplements companies to select the proper one, specifically focusing on the purity of luteolin supplement. However, many dietary supplements on the market have different luteolin formulations that can be confusing and misleading. It is crucial to choose a formulation with high purity luteolin in order to experience any noticeable benefits [31]. For these reasons, we chose the luteolin supplement from the SuperSmart Company, which has an 80% purity level in Veggie capsules.

In a rat model, we designed the study to detect the oxidative disorders of nicotine injection and evaluate the potential protective role of luteolin and quercetin supplements single or combined to overcome the oxidative deterioration intra- and intercellular.

During the experimental time of 8 weeks, the findings of all weight parameters indicated that the nicotine injection had a worse effect on all body and organ weight parameters. The noticeable weight reduction in the Ni group might be due to a decrease in appetite linked to nicotine injection. This aligns with earlier studies indicating how nicotine affects the satiety center in the hypothalamus, leading to reduced food consumption [32]. **N**icotine exposure impact on changing eating habits, may possibly due to the heightened energy expenditure caused by nicotine [32]. The regulation of appetite and body weight by nicotine is frequently highlighted as a main factor motivating smoking initiation amongst young individuals, particularly adolescent girls [33]. Recent research indicates that inhaling nicotine boosts locomotor activity in both sexes of rats to a comparable or even greater extent than subcutaneously injected nicotine [34]. Few studies didn`t record significant loss in weight within nicotine administration, may be because of many reasons including animals age and sex, low nicotine dose, and short experimental period [23, 35]. In the current study, liver, lungs and brain weights and relative weight in Ni-treated rats were significantly decreased compared to other experimental groups. Our findings were harmonious with former researches on liver and lung of mice exposed to negative smoking [36], on rabbit liver [37]. The liver is the primary location for nicotine metabolism, although the lung and kidney also play a role in this process [8]. Nicotine has a significant influence on the developing fetal brain by easily and quickly crossing the placenta [38]. Few studies disagreed with our findings [39]. Meanwhile, rats treated with Qu and/or Lu coupled with Ni, showed reversed

effect on all body and organ weight parameters. Former studies have documented Lu`s hepatoprotective ability in cases of liver injury [22, 40]. The notable enhancement that appear in Qu supplement group was in line with previous literatures [20, 21]. Generally, the Lu and Qu supplement combined showed a synergetic effect in all weight parameters, which were in agreement with studies using the same combination [41], Qu combined with vitamin C [21], Qu with curcumin in liver dysfunction [42].

The present study declared that, injection of nicotine caused considerable increases in liver function enzymes (ALT, AST and LDH) activity compared to the control and supplemented groups. These elevations indicating impairment in liver function of Ni injected rats. Our findings consistence with previous studies used nicotine dosage (1 mg/Kg) for 6 weeks in adult male mice [43], and s.c. injection (4 mg/Kg) in male F-344/NHsd Fisher rats [44]. A recent study reported that nicotine injection in different doses could influence liver enzyme function (ALT and AST) in neonates on postnatal days 7, 14 and 21 [45]. The increment in liver function enzyme activities (ALT and AST) in serum of Ni group could be attributed to ultimate hepatocellular injury resulted from oxidative stress caused by nicotine [44]. Our findings detected that Qu supplement group showed the best improvement of all liver function enzymes against drastic increases that induced by nicotine. There is evidence to suggest that quercetin has the potential to protect the liver from harm caused by hepatotoxins [41]. Luteolin supplement treated group showed less improvement on liver function enzymes than Qu supplement, may be because the relatively low bioavailability of luteolin suggests using modified forms or derivatives to enhance its bioavailability [40]. Recent study reported that quercetin showed a perfect antioxidant and anti-inflammatory activities using phagocytosis, tests of radical scavenging activity. Quercetin activity is directly proportional to its five phenol (OH) groups [46].

Studies on human and animals have shown a link between fatty liver, oxidative stress and lipid peroxidation [47]. Nicotin injection led to considerable increment of MDA value, but considerable decrement in CAT, SOD, GPx and GSH contrasted with other experimental groups. Our findings were compatible with previous studies [39, 48]. Recent study reported higher levels of MDA in neonates of mothers injected with different doses of nicotine at postnatal days 7, 14 and 21, accompanied by notable decreases in CAT, SOD and GSH activities [45]. Endogenous antioxidant enzymes serve as the main line of defence against oxidative damage to biological macromolecules. Nonetheless, nicotine interferes with the mitochondrial respiratory chain, causing a rise in the production of superoxide anions ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), which leads to a reduction in cellular antioxidants [39, 45]. Former study reported that nicotine might cause activation of nuclear factor-kB pathway resulting in the releasing of C-reactive protein (CRP), that can trigger the generation of reactive oxygen species (ROS) and elevate the level of MDA [49]. Elevated levels of the lipid peroxidation marker MDA can hinder the proper functioning of hepatocyte membranes, leading to the release of cytoplasmic markers like LDH into the bloodstream as a signal of cell damage [21]. A reduction in GSH level in rat exposed to nicotine suggest that GSH helps to protect cell membrane from oxidative damage by capturing free radicals and transitioning from a reduced to oxidized state [47]. Nicotine-induced oxidation in serum can cause inactivation of SOD and CAT proteins, or reduce their endogenous synthesis. The diminished SOD activity can be attributed to its interaction with $O_2^{\bullet-}$ to generate H_2O_2 and O_2. Additionally, H_2O_2 is CAT enzyme substrate, which works to break down H_2O_2 into water and oxygen [50], thereby reducing its efficacy, as shown in our results. Current study revealed that administration of Lu alone and in combination with Qu can significantly restored all enzyme activities to normal levels, as well as reduced MDA to minimum level. We suggest that Lu and Qu suppressing ROS production and/or boost cellular antioxidant defences. Many literatures indicated that Qu and Lu have antioxidant properties, scavenge free radicals, and provide protection against radiation [42, 46, 50]. The antioxidant efficacy of Qu and Lu may be attributed to (i) their increased penetration into cell membrane, enabling them to neutralize free radicals and protect the membrane from harm [18, 50]; (ii) their structure contains 4 and 5 hydroxy group that give them an ability to chelate metal ions, thereby scavenging lipid alkoxyl and peroxyl radicals [15, 47]. Moreover, they can promote the upregulation of these enzymes by influencing nuclear factor erythroid2-related factor (Nrf2), which regulates the transcription of various genes, including detoxifying enzymes, through antioxidant-responsive elements (ARE) [51].

Our results detected significant DNA damage rate on serum, lungs and liver tissues in Ni group rats. Our data are in consistence with other published data in rat alveolar type II lung cells [52], and recently on mice lung and liver in comparison between e- and ordinary cigarettes smoking [53]. Many mutagenic and genotoxic by-products of lipid peroxidation, including MDA have been found to bind to DNA and cause damage [12]. This could potentially explain the heightened level of DNA damage observed in cells treated with nicotine in our study. We found that DNA damage was considerably diminished in Lu and Qu combined group, which showed no fragmentation on gel electrophoresis similar to control in all studied samples. There are many studies that demonstrated

the effectiveness of Quercetin and luteolin in protecting DNA from oxidative damage induced by smoking, drugs or radiation in animal models [19, 54, 55]. In preclinical studies, quercetin and luteolin protect DNA from oxidative harm by regulating lipid peroxidation and enhancing the antioxidant defence system. In clinical studies, it was found that Qu and Lu supplementation increase antioxidant capacity and reduce the risk of diseases. [16, 40].

Conclusion

Briefly, nicotine injection in young rats caused significant loss in weight gain and organ relative weight. Additionally, impairment in liver function and redox imbalance state. In the cellular level, nicotine caused significant DNA fragmentation (damage) in serum and both liver and lung tissues. The combination of Qu and Lu supplements appears to effectively enhance the antioxidant defences, restore the adverse effects of nicotine in weight parameters, liver function and protecting DNA from oxidative damage. This work is one of few studies that applied a combination of quercetin and luteolin supplements to evaluate their synergetic effects as cellular protectors against passive smoking harmful subsequences.

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Declaration of Conflict of Interest

The authors declare that they do not have any conflicts of interest pertaining to this study.

Ethical of approval

All experimental protocols were approved by Research Ethics Committee of King Abdulaziz University, Saudi Arabia, under the project of the research number of (1-17-04-009-0007).

TABLE 1. Effect of nicotine (Ni) and supplements administration on final rat weight, weight gain and percentage of weight gain of young rat

Experimental Groups	Initial wt (g)	Final wt (g)	Weight gain (g)	Weight gain (%)
Cont	66.67±1.82	194.67±6.92	127.36±6.54	65.16±1.3
Ni	69.73±2.39	143.33±6.42^{a**}	74.02±7.9^{a**}	50.57±3.5 ^{a**}
Ni+Lu	67.12±2.48	208.0±11.09^{b**c*}	139.92±11.2^{b**,c*}	66.57±2.2 ^{b**}
Ni+Qu	68.17±2.31	178.33±7.94^{b**}	110.33±8.16^{b*}	61.24±2.36 ^{b*}
Ni+Qu+Lu	66.49±1.24	195.44±7.64^{b**}	128.72±6.8^{b**}	65.52±1.2 ^{b**}

Data are presented as mean ± SE (n=10).
Cont: normal control, Ni: nicotine, Lu: luteolin, Qu: quercetin.
[a] Significant difference versus Cont, [b]: Significant difference versus Ni, [c]: Significant difference versus Ni+_Ou, [d]: Significant difference versus Ni+Lu. ($P \leq 0.05$*, $P \leq 0.01$**).

TABLE 2. Effect of nicotine (Ni) and Quercetin (Qu) and luteolin (Lu) supplements on serum liver-function enzymes of young rats.

Experimental Groups	ALT (U/L)	AST (U/L)	LDH (nmol/min/mL)
Cont	18.04 ±1.4	48.31±5.32	43.6 ± 1.43
Ni	60.65 ± 8.49 ^{a**}	98.07±4.82^{a**}	144.4± 19.8 ^{a**}
Ni+Qu	17.34 ± 0.77^{b**}	37.6±3.7 ^{b**}	51.6 ± 3.44 ^{b**}
Ni+Lu	35.94 ± 0.59 ^{a*,b**,c*}	55.45±1.8 ^{b**,c*}	80.6 ± 4.9 ^{a*,b**,c*}
Ni+Qu+Lu	31.56 ± 2.84 ^{a*,b**,c*}	58.33±3.4^{b**,c*}	85.4 ± 1.7 ^{a*,b**,c*}

Data are presented as mean ± SE (n=10).
ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase
[a] Significant difference versus Cont, [b]: Significant difference versus Ni, [c]: Significant difference versus Ni+Ou, [d]: Significant difference versus Ni+Lu. ($P \leq 0.05$*, $P \leq 0.01$**).

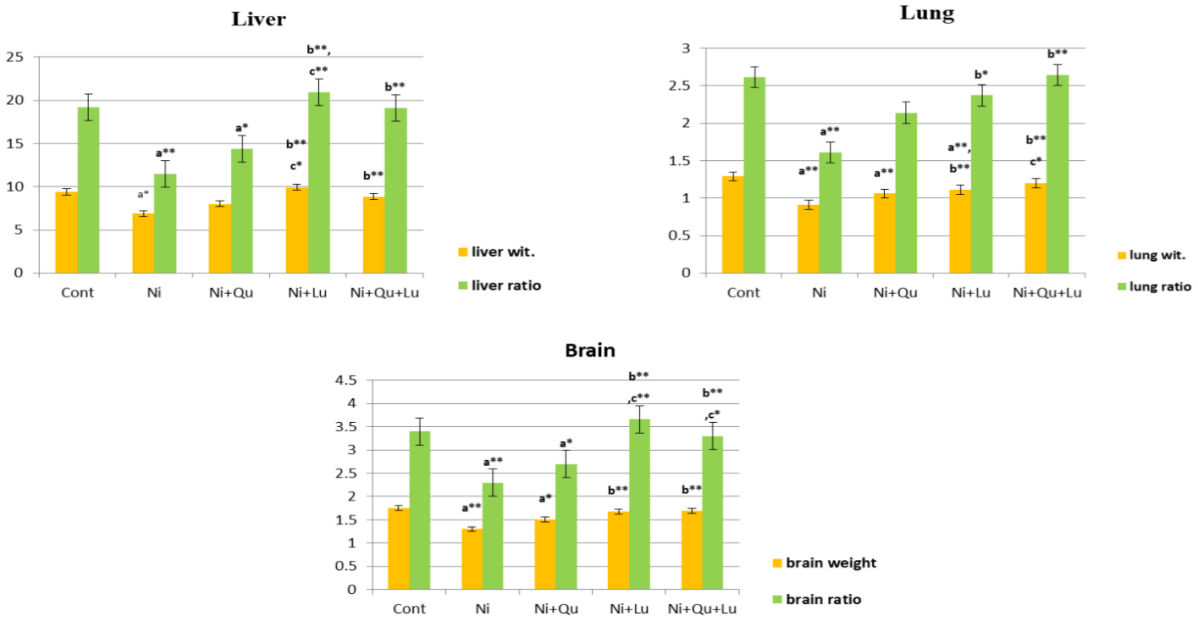

Fig. 1. Effect of nicotine (Ni), Quercetin (Qu) and Luteolin (Lu) on weight and relative wt ratio of liver, lungs and brain of young rat groups.
Cont: normal control, Ni: nicotine, Lu: luteolin, Qu: quercetin.
[a] Significant difference versus Cont, [b]: Significant difference versus Ni, [c] Significant difference versus Ni+Qu [d]: significant difference versus Ni+Lu. (P ≤ 0.05*, P ≤ 0.01).**

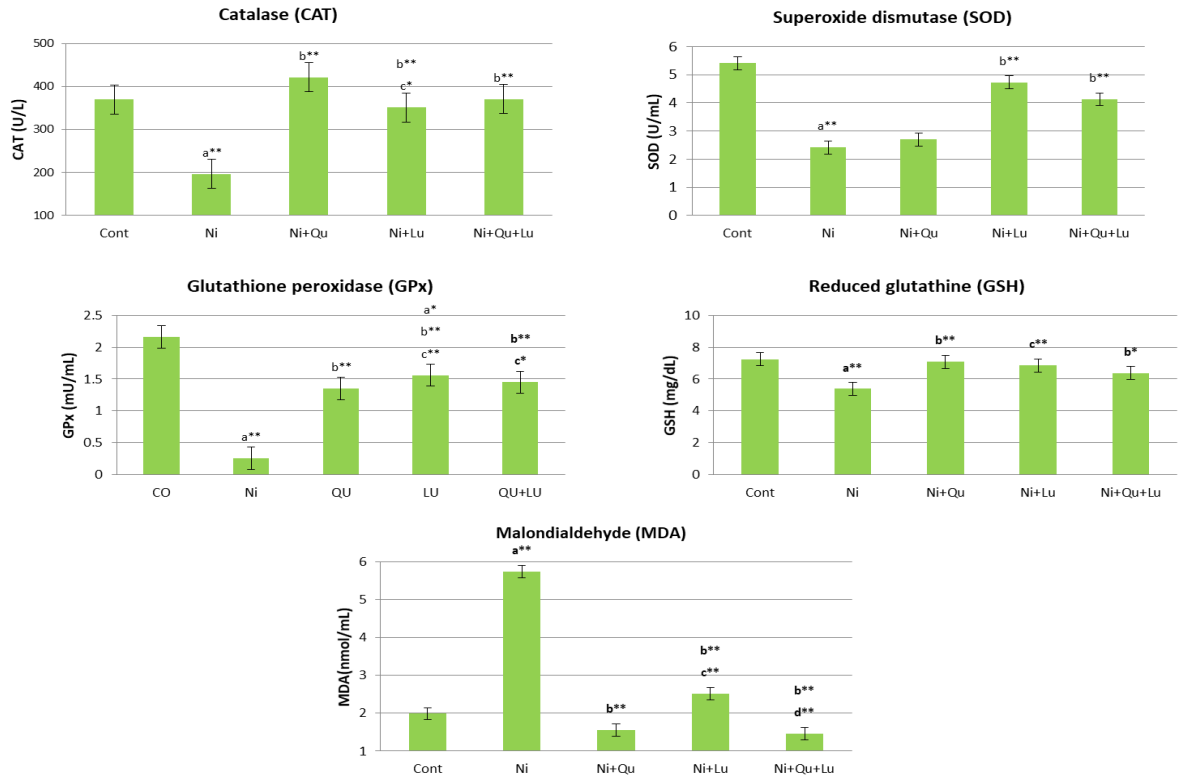

Fig. 2. Effect of nicotine (Ni), Quercetin (Qu) and Luteolin (Lu) on serum antioxidant markers CAT, SOD, GPx and GSH levels, MDA in young rat groups
Cont: normal control, Ni: nicotine, Lu: luteolin, Qu: quercetin.
[a] Significant difference versus Cont, [b]: Significant difference versus Ni, [c]: Significant difference versus Ni+Ou, [d]: Significant difference versus Ni+Lu. (P ≤ 0.05*, P ≤ 0.01).**

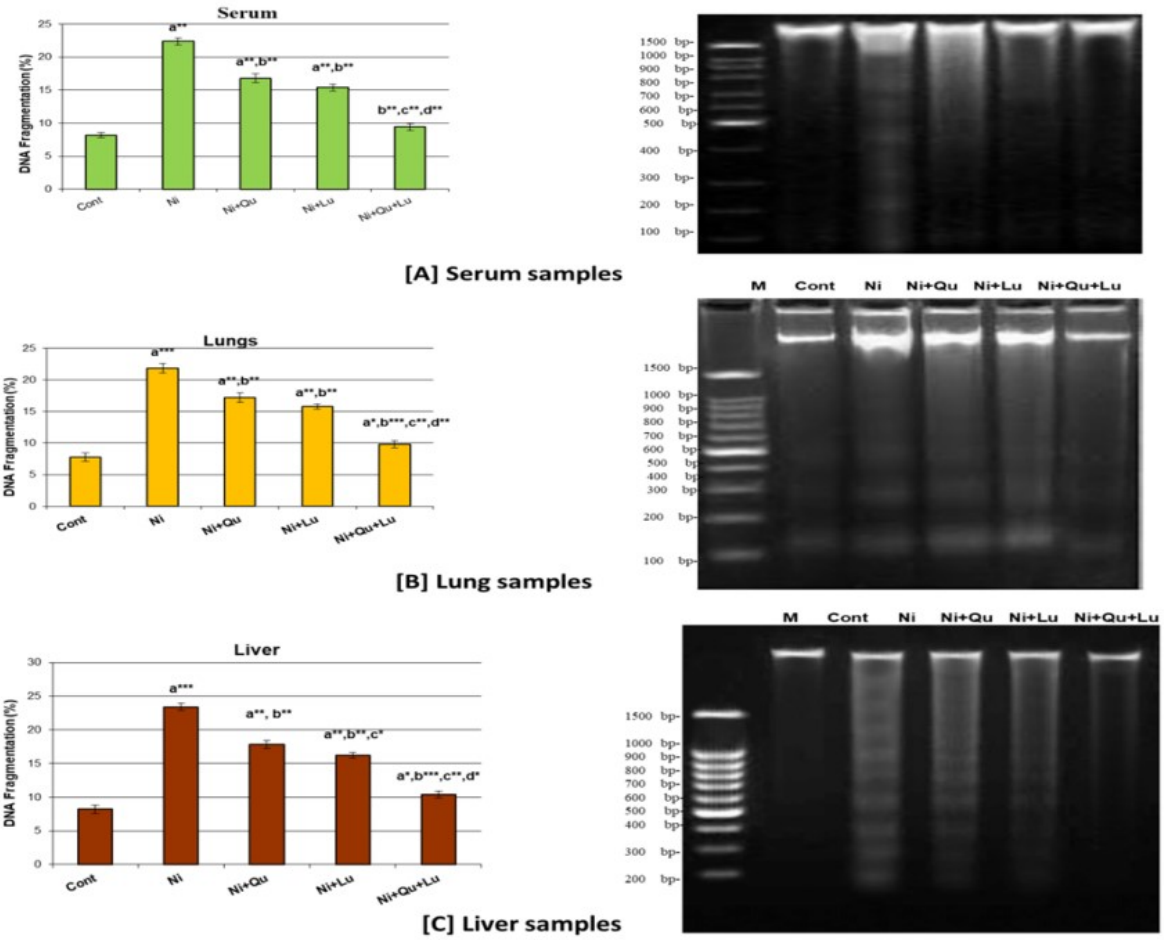

Fig. 3. The effect of nicotine and supplements on DNA fragmentation of (A) serum, (B) Lungs, and (C) Liver tissues of young rat groups. (left side) analyzed by diphenylamine reaction procedure and (right side) detection with agarose gel electrophoresis.
Cont: normal control, Ni: nicotine, Qu: quercetin, Lu:luteolin, M: DNA ladder
[a] Significant difference versus Cont, [b]: Significant difference versus Ni, [c]: Significant difference versus Ni+Ou, [d]: Significant difference versus Ni+Lu. (P ≤ 0.05*, P ≤ 0.01).**

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التأثير الوقائى للمكملات الغذائية الليتيولين والكيرستين على الجهد التأكسدى وتلف الحمض النووي الناجم عن التعرض للنيكوتين في صغار الفئران

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الملخص

مقدمة:

يشكل التدخين السلبى خطرا كبيرا على صحة الأطفال، حيث قد يؤدى الى زيادة فرص اصابتهم بالأمراض والتي قد تصل الى الوفاة.

هدف الدراسة:

تهدف هذه الدراسة الى تقييم التأثير الوقائى لتناول مدعمات الليتيولين والكيرستين لحماية الجسم من الأكسدة وتلف الحمض النووي الناجم عن التعرض للنيكوتين في الفئران صغيرة العمر. بالإضافة الى تحديد تأثير الليتيولين ومقارنته بمدعم الكيرستين.

تصميم التجربة :

شملت التجربة البيولوجية خمس مجموعات من الفئران (المجموعة 10 فئران) حيث تم تقسيمهم كما يلى: المجموعة (1) مجموعة ضابطة – المجموعة (2) تم حقنها بالنيكوتين تركيز (0.75 مللجم/كجم وزن الفأر) – مجموعة (3) تم حقنها بالنيكوتين بالإضافة الى مكمل الكيرستين بتركيز (50 مللجم/كجم وزن الفأر)- مجموعة (4) تم حقنها بالنيكوتين بالإضافة الى مكمل الليتيولين بتركيز (50 مللجم/كجم وزن الفأر) – المجموعة (5) تم حقنها بالنيكوتين بالإضافة الى مزيج من الكيرستين والليتيولين (50 مللجم/كجم وزن الفأر) لكل منهما. بعد 8 أسابيع (المدة الزمنية للتجربة) تم جمع عينات مصل الدم و الكبد وكلا الرئتين.

النتائج:

أظهرت معايير وزن الجسم للفئران انخفاض كبير معنوى احصائيا في فئران مجموعة النيكوتين (مجموعة 2) مقارنة بالمجموعة الضابطة والمجموعات الأخرى. حدث زيادة كبيرة معنوية في مستويات نشاط إنزيمات وظائف الكبد و تركيز مركب المالوندايالدهيد في فئران مجموعة النيكوتين (2) مقارنة بالمجموعة الضابطة (1). في حين حدث انخفاض ملحوظ فى مستويات نشاط انزيمات الأكسدة الذاتية في فئران مجموعة النيكوتين(2). أظهرت فئران مجموعتى الليتيولين منفردا (4) و مزيج الليتيولين مع الكيرستين (5) تحسنا ملحوظا في جميع المؤشرات السابق ذكرها مقارنة بمجموعة النيكوتين منفردا (2). على المستوى الخلوى، حدثت زيادة في تلف الحمض النووي بشكل ملحوظ في مصل الدم والكبد والرئتين في مجموعة النيكوتين (2) ، في حين لم يظهر هذا التلف في الحمض النووي في فئران مجموعة المزيج بين الليتيولين والكيرستين (5).

الاستنتاج:

كان التأثير الوقائى لمدعم الليتيولين مماثلا لتأثير مدعم الكيرستين للوقاية من الأكسدة الناتجة من النيكوتين. تشير البيانات الى ان مدعم الليتيولين يؤدى تأثيره الوقائى من خلال تعزيز آلية الدفاع المضادة للأكسدة وتعديل مستويات أكسدة دهون الجسم وبالتالي حماية الحمض النووي من التلف في مصل وأنسجة الفئران. وقد أدى استخدام مزيج من المكملين الغذائيين الى ظهور التأثير التآزرى لخصائصهما المضادة للأكسدة ضد الجهد التأكسدى الناتج من التعرض للنيكوتين.

الكلمات الدالة : نيكوتين ، الجهد التأكسدى ، ليتيولين ، كيرسيتين ، تلف الحمض النووي.