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Ameliorative Effect of Quercetin and Luteolin Supplements on Histology of Liver and Lungs Intoxicated with Nicotine in Young Rats

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ABSTRACT

Nicotine is a potential inducer of oxidative stress, which can damage the numerous tissues and biological molecules. Nicotine is first metabolized in liver, and the lung is the main target organ susceptible to smoking; therefore, the purpose of the current examination was scrutinizing the impacts of nicotine on these organs using the histological method, and studying the ameliorative (additive or synergistic) effects of quercetin and luteolin supplements in target tissues against nicotine toxicity in young albino rats. Fifty animals were involved in this study which were divided into five groups of ten rats, they were treated as: (1) untreated Control (Cont); (2) Nicotine (Ni) treated (0.75 mg/kg b.w./d, i.p.); (3) Nicotine as above with intragastric administration of quercetin (Ni+Qu) (50mg/kg b.w) or (4) with luteolin (50mg/kg b.w) in Group (Ni+Lu), and (5) nicotine with combination of quercetin and luteolin (Ni+Qu+Lu) as described above. All groups were treated for 8 weeks, then their liver and lung tissues were dissected out. Several histological damages were noticed in (Ni) rats such as degeneration changes in hepatocytes, slight congestion of central vein (CV) and cellular infiltration. Nicotine caused drastic changes in lung tissues such as, inflammatory cells aggregation inside and outside the bronchiol artery with mild increase in the thickness of bronchial muscular wall and marked thickening in the alveolar wall. All supplemented groups ameliorated the damage that induced by nicotine injection in both liver and lung tissues. In liver tissues, the quercetin (Ni+Qu) supplemented rats showed the better improvement nearby normal hepatocytes than the other groups, while in lung tissues, the luteolin supplemented rats had better recovery than the other treatments. These observations suggested that the intake of quercetin and luteolin as supplements may be useful in combating tissue injury that is a result of nicotine toxicity.

Key words: *Nicotine, quercetin, luteolin, toxicity, supplements.*

INTRODUCTION

Smoking has enormous negative health consequences worldwide, and the use of tobacco is still rising globally [1]. Cigarette smoking and passive exposure to cigarette smoke are risk factors which produce many chronic diseases which increase morbidity and mortality [2]. A previous research reported that one cigarette reduces your life by 11 mins [3]. It has become clear that health threats are particularly serious for children, who constitute a vulnerable population that cannot voluntarily avoid secondhand smoke (SHS) [4]. Yearly about 150,000 – 300,000 children <18 mon old are exposed to negative smoking. Those children are at high risk to severe asthma, respiratory tract infections and death [5, 6]. The Saudi Arabia is ranked 8th in the world in terms of tobacco consumption [7]. In the years of 2001 to 2010, the economic burden of tobacco consumption in the KSA was 20.5 billion US dollars [1]. Each cigarette contains about 10 milligrams of nicotine [8].

Nicotine is a natural alkaloid obtained from the leaves and stems of the *Nicotianatabacum* and *Nicotianarustica*. [9]. Liver oxidizes 80-90% of nicotine to its main metabolite cotinine that causes the formation of free radicals in tissues [10]. Nicotine and its metabolites induce oxidative stress both *in vitro* and *in vivo*, and contributes

with a major proportion to the net oxidative stress imposed by tobacco use, and at the same time, depletes the antioxidant defense mechanisms [11].

Antioxidants in the living body play an important role in the defense system against oxidative stress. However, the innate oxidant defense system is not sufficient to adequately deal with the amount of reactive oxygen species produced, and it is now thought that dietary antioxidants are essential for health and well being [12]. Consuming fruits and vegetables rich in antioxidants, including flavonoids - polyphenolic compounds common in fruits, and vegetables can strengthen the defense system [13]. Flavonoids is considered as powerful antioxidants and anti-inflammatory in numerous studies [14, 15]. The Quercetin from flavonols group and Luteolin from flavones group are mostly found in many vegetables and fruits, which possess antioxidant properties [16, 17]. They are consumed in a too low level in a way that their effects can not be clinically meaningful but when they are consumed as supplements, their protective effects can be easily be expanded [18]. This study aimed to detect the effectiveness of Quercetin and Luteolin supplements individually or in combination to alleviate the disorders that induced by nicotine in liver and lung tissues in young Wistar albino rats.

MATERIAL AND METHODS

Chemicals

Nicotine hydrogen tartarate was obtained from Glentham Life Science (England). Nicotine stock solution was prepared at concentration of 2 mg/mL in normal saline solution and stored in 4 °C. Phosphate Buffered Saline (PBS) was obtained from LonzaWalkerville (USA), and Dimethyl Sulfoxide (DMSO) was obtained from Biomatik (Ontario, Canada). The Quercetin and Luteolin supplements were obtained from GNC (USA) and Supersmart (France); respectively.

Experimental design

All the experimental procedures were approved by the University Ethics Committee for Animal Experimentation. Fifty young male Wistar rats, with an average weight of 70-80 g were used. Before starting the experiments, the animals were placed in plastic cages under the standard laboratory conditions (temperature 22±2 °C; 12:12 h light:dark cycle) for at least one week to adapt. During the experiments, tap water and commercial standard rodent laboratory diet were available *ad libitum*. The rats were randomly divided into five groups (n=10 per group) and treated for 8 weeks, as follows: (1) the control group (Cont) was receiving intraperitoneal (*i.p.*) injections of normal saline (0.1 mL/ kg body weight (b.w.)); (2) nicotine group (Ni) received nicotine only (0.75 mg/kg (b.w.) per day (i.p.)); (3) and (4) the nicotine + quercetin (Ni-Qu) and nicotine + Luteolin (Ni+Lu), respectively received nicotine as described above with quercetin or luteolin (50 mg/kg (b.w.) per day intra-gastric); and (5) the nicotine + combination of quercetin and luteolin (Qu 50 mg/kg and Lu 50 mg/kg (b.w.) per day i.g). These doses were chosen according to the studies done before [16, 19, 20] and were related to the human daily dietary intake of quercetin enriched food and the daily nicotine intake in people who smoke 10-20 cigarettes per day [9]. The nicotine dosage was freshly prepared in normal saline solution, then intraperitoneally injected once a day for 8 weeks at 11.00 AM. Whereas, the quercetin and Luteolin supplement dosages were weekly prepared in 1% DMSO in PBS solution, then administered orally by cavage once a day for 8 weeks consecutively, one hour before nicotine injection (at 10.00 AM) [16].

Preparation of tissues for light microscopy

Twenty-four hours after the last dose, the rats were sacrificed by ether anesthesia, and the tissues of interest liver and lungs were gently dissected out, washed well with normal saline (0.9% NaCl), and placed in 10% neutral buffered formalin for at least 24 hrs. To ensure the adequate fixation, the formalin was used at a volume 10-20 times more than the volume of tissue pieces. The fixed tissues were then trimmed, washed with ice saline and dehydrated. The Dehydration was achieved by passing tissues through ascending grades of isopropyl alcohol followed by two changes of xylene. After infiltration in paraffin wax, the tissues were embedded in paraffin wax [21]. The paraffin blocks were cut with rotary microtome (Spencer 50) at almost 5µm thickness. The sections were floated on a tissue floatation bath at 40 ºC and taken on glass slides. The sections were then melted for 5 min in an incubator at 60 ºC, then kept for cooling and stained with hematoxylin and eosin [22]. The sections were examined and photographed using an optical photomicroscope (Nikon U-III Multipoint Sensor System, Japan).

RESULTS

Liver

Examination of H & E sections of the liver at central vein (CV) region of the control group (Cont) showed normal hepatocyte cell cords radiating from CV, with indication of normal active status showed as round and light stained cell nuclei. There was normal appearance of blood sinusoids between hepatic cells (Figure 1a). While the liver sections of nicotinic group (Ni) showed damage and slight congestion of CV outlined with disorganization of normal hepatocyte radial arrangement. Most of hepatocyte nuclei are small and dark, and the cells' cytoplasm was stained and darker, indicating degeneration. It also showed an irregularity of blood sinusoids between cells (Fig. 1b-1). In other regions of liver, Ni rats showed massive hepatocyte necrosis and dark degenerated nuclei (Fig. 1b-2). The liver sections of Ni+Qu rats showed nearly normal structure of CV or hepatocyte with mostly rounded active nuclei (Fig. 1d). While in liver section of (Ni+Lu) rats, there was a moderate protection with mild necrosis on some regions and dark nuclei in numerous cells (Fig. 1e). When two supplements were combined in (Ni+Qu+Lu) group, the sections showed potential protection compared to Ni rats, but there was still mild congestion with dark degenerated inactive nuclei of hepatocytes (Fig. 1f). The histological examination of liver sections of all supplemented groups showed that the better improvement of liver CV and hepatocytes was observed in the nicotine with Quercetin supplemented rats through the 8 weeks of the experimental period.

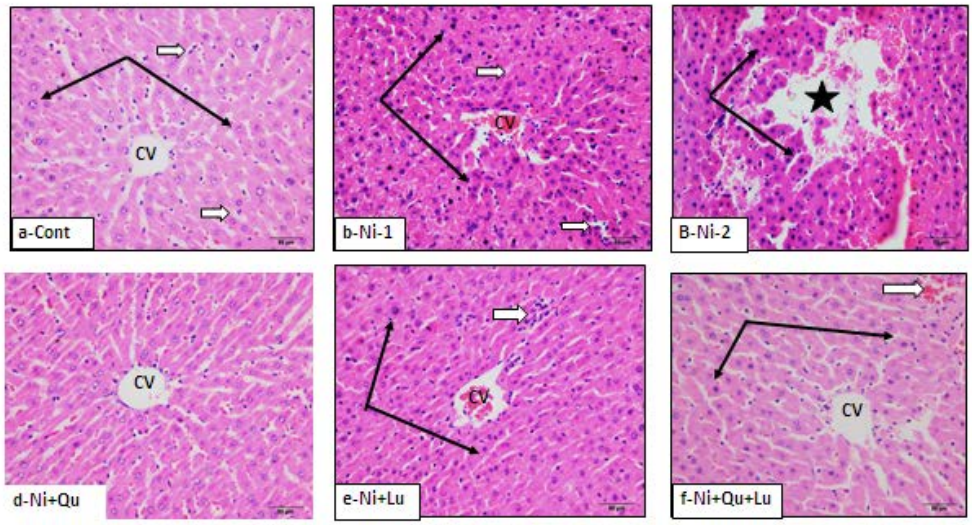

Figure 1: Effect of nicotine and quercetin and luteolin supplements on sections from rats liver central vein (CV) stained by H&E dyes.

a) Cont: with normal hepatocyte cell cords (black arrows), radiating from CV. Blood sinusoids between cells are of normal appearance (white arrows). **b-1) Ni:** Showed damage and slight congestion of CV outline with disorganization of normal hepatocyte radial arrangement (black arrows). Most hepatocytenuclei are small and dark indicating degeneration, and cytoplasm is also stained and darker. Blood sinusoids between cells are compressed or irregular (white arrows). **b-2) Ni:**Other regions showed massive hepatocytenecrosis (stars) and nearby cells showed dark degenerated nuclei (arrows). **d) Ni+Qu:** Nearly normal structure of CV or hepatocyte (arrows). Most hepatocytes have rounded active nuclei (lightlystained). **e) Ni+Lu:** Moderate protection with mild necrosis (white arrow) in some regions and dark nuclei in numerous cells showed dark nuclei (black arrows). **f) Ni+Qu+Lu:** still mild congestion (white arrow) with potential protection and hepatocytes with dark degenerated inactive nuclei (black arrows)

Lung

Lung bronchiole and bronchiole artery sections of Cont rats group showed opened lumen with notice of thin muscle layer around the bronchial wall and normal thickness bronchial arteriole. It was also noticed that the surrounding alveoli and bronchial artery had thin wall (Fig. 2a). While the lung sections of rats injected with nicotine (Ni) were found with folded hypertrophied epithelial lining, and cell debris and inflammatory cells

were noticed within the lumen. Similar inflammatory cells aggregation were seen outside the wall closing nearby alveoli with a mild increase thickness of bronchial muscular wall (Fig. 2b). In Ni+Qu supplemented rats, the lung sections of bronchiole showed the marked preservation of bronchial epithelium, which appeared nearly as a normal epithelium. The residual of few cell debris in lumen with improvement in muscle thickness was also shown. Few scattered inflammatory cells were noticed around the bronchial wall, while nearby alveoli were patent with wide, and free of inflammatory cells (Fig. 2c). In Ni+Lu supplemented rats, the lung sections showed the same improvement that was noticed in Ni+Qu rats with bronchiole lumen free of any cell debris and less inflammatory cell aggregation around bronchiole. Also, the similar improvement was found in alveoli and bronchial artery thick wall that appearred in Ni+Qu (Fig. 2d). In Ni+Qu+Li rats' lung sections, there was the same level of improvement in bronchiole`s epithelium and lumen and alveoli (Fig. 2e). The better improvement was noticed in Luteolin (Ni+Lu) and quercetin and luteolin combined (Ni+Qu+Lu) supplemented rats more than Ni+Qu rats.

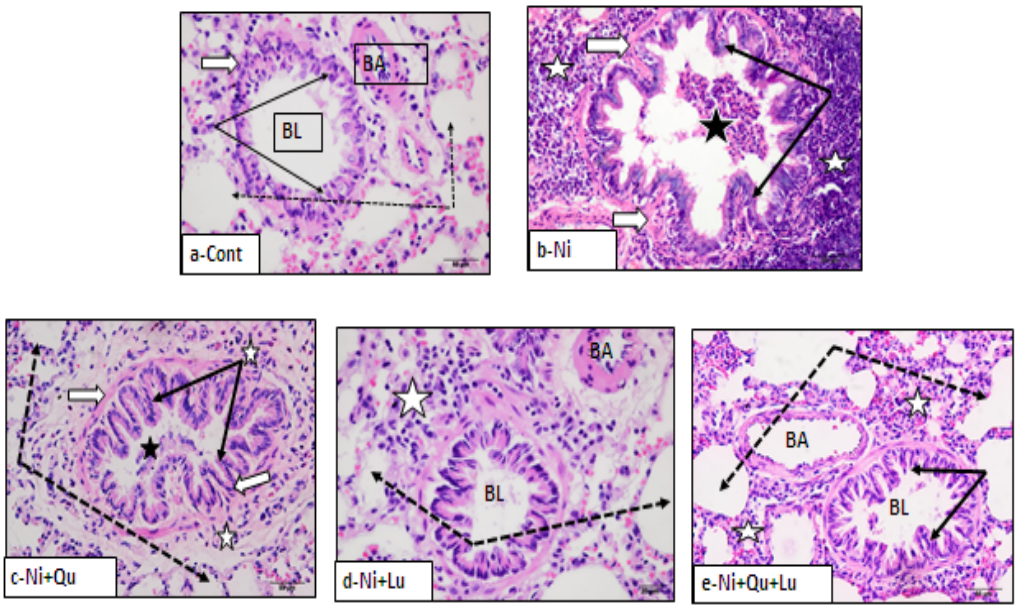

Figure 2: Effect of nicotine and quercetin and luteolinsupplements on sections of young rats' lung bronchiole and bronchiole arterythatstained by H&E dyes.

a) Cont: Bronchiole with patent opened lumen (BL) and lining epithelium (black arrows), with its accompanied normal thickness bronchial arteriole (BR). The surrounding thin wall patent alveoli (dotted arrows). The thin muscle layer around the bronchial wall (white arrow) and Bronchial artery has a thin wall.(BA). b) **Ni:** Bronchiole with folded hypertrophied epithelial lining (arrows). Cell debris & inflammatory cells within the lumen (black star). Aggregation of inflammatory cells (white stars). A mild increase in thickness of bronchial muscular wall (white arrows). c) **Ni+Qu:** The normal epitheliumin side bronchiole (black arrows), residual of few cell debris in lumen (black star), and improved muscle thickness (white arrows). Few scattered inflammatory cells (white stars) around the bronchial wall. Nearbyalveoli are patent with wide and free of inflammatory cells (dotted black arrows). d) **Ni+Lu:** Bronchiole lumen free of anycelldebris (BL). Inflammatory cell aggregation around is less (white star). Alveoli are patent and have wide lumina (dotted black arrows), Bronchial artery has a thinner wall (BA). e) **Ni+Qu+Lu:** Bronchiole with a preserved lining epithelium (black arrows). The lumen is patent and free of celldebris (BL). Mild inflammatory aggregation between alveoli (white stars). Most nearby alveoli are patent with wide lumina (dotted arrows).

DISCUSSION

The findings of histological examination of (1) the liver tissue revealed the marked tissue damage in nicotine treated group compared to control, (2) the lung tissue revealed the hypertrophy of bronchiole artery with many aggregations of inflammatory cells related to toxicity of nicotine treatment, (3) supplementation with quercetin

and luteolin individually or in combination markedly preserved the damage that was induced by nicotine treatment in different levels. The findings of this study admitted the expectations which were based on the previous studies about the prooxidative effects of nicotine, and the antioxidative effects of quercetin and Luteolin [16, 19, 23-25].

Cigarette smoking is popular globally, and has been proven to be hurting human health specially children. It increases the risk of developing chronic liver diseases independent of the liver status [26]. Nicotine, which is a main toxic element existing in cigarette smoking [27] quickly absorbed through the lung, and is majorly metabolized in the liver [28]. Administrating nicotine in rats chronically, results in cytochrome P-450, produces free radicals in tissues, and causes oxidative tissue injury [29]. The liver is a major organ for drug biotransformation, therefore is highly susceptible to the oxidative events associated with the toxicity of nicotine [26]. The histological tests of this study detected damage and slight congestion of CV, outlined with disorganization of normal hepatocyte radial arrangement. Also the degeneration of most hepatocyte nuclei with compressed or irregular blood sinusoids between hepatocytes was found. All supplemented groups of rats showed good and moderate protection, while the better improvement of liver CV and hepatocytes was observed in the nicotine with Quercetin (Ni+Qu) supplemented rats through the experimental period.

The findings of this study were consistent with [30], who detected histological changes in normal liver cells of albino rats after being exposed to cigarette smoke for 90 days. They detected a mitochondrial crowd in liver cell cytoplasm associated with proliferation of the endoplasmic reticulum. Chronic nicotine exposure dose (2.5 mg/kg b.w by s.c) of male Wistar rats for 6 weeks showed morphological alteration in liver tissue compared to the control [31]. Similar findings were detected but with nicotine dose of (1 mg/kg b.w./d, s.c) for 21 days in Wistar rats [32]. In the current study, Quercetin group rats (Ni+Qu) showed the better recovery of hepatocytes and CV from all supplemented groups, because of the reason that quercetin (flavonol) has multiple beneficial health effects including antioxidant properties [33]. There is a reason to believe that quercetin can shield the liver from damage brought about by hepatoxins [34]. Quercetin had an ameliorative effect as an antioxidant on toxicity that was induced by nicotine in HepG2 cells [35]. Luteolin (flavones) is a food-derived compound, and possesses multiple pharmacological activities [36].

Recent study suggested that luteolin protected against HgCl2-induced liver injury, and its protection mechanism depends on its ability to decrease oxidative stress parameters in rat model [37]. Nicotine consumption induced an inflammatory response in the lung, and played a role in pathogenesis of obstructive pulmonary diseases [38]. An aggregation of inflammatory cells was detected on bronchiole lumen and outside the wall that closed nearby alveoli with a mild increase thickness of bronchial muscular wall that was initiated by nicotine injection. These structural changes recovered in different degrees in supplemented groups with better improvement being noticed in Luteolin supplemented group (Ni+Lu).

The findings of this study were similar to a previous research study done by [39], who used the same concentration of nicotine (1 mg/kg/d for 8 days) but in adult male Wistar rats (3-4 months). Their treatment exhibited an increase of the volume fraction of alveolar parenchyme, thickening of the alveolar septa, and was associated with mononuclear cell infiltration, angiogenesis, and irregular areas of collapse compared to the control ones. A recent study detected marked inflammatory histological damages on liver and lung tissues of adult male Wistar albino rats exposed to nicotine (1mg/Kg b.w/d, s.c.) for 21 days [32]. In addition, several studies reported the harmful damages of lung tissue due to smoking. A recent study reported the alveolar morphological damage by third hand smoking (THS) on weaning C57BL/6 mice (aged 3 w) for 6 months. They also detected cellular infiltration in some areas of the respiratory bronchioles of the THS-exposed mice; whereas there was none in controls [24]. While [40] detected marked thickening in the alveolar wall, collapsed alveoli, blood extravasations and inflammatory cell infiltration. In addition, there were drastic histological changes in the tracheal epithelium of albino rats exposed to cigarette smoking for 3 months. The histological findings on Ni rat lungs indicated that nicotine induced changes similar to those of chronic irritation of tissue might decrease the efficiency of gaseous exchange in alveoli, and predispose to neoplastic changes [40]. Also, an increased risk for development of fibrosis was suggested in young people who have been exposed to nicotine for prolonged periods of time as TSH [24].

Nicotine treatment caused an accumulation of inflammatory cells in the lung tissues in the present study. These inflammatory cells might contribute in damaging the alveolar and interstitial pulmonary structures through secretion of lytic enzymes and oxygen free radicals [41]. There were some limitations to this study: the protective effects of quercetin or luteolin supplements and their combination were evaluated against nicotine alone, as only one of the many components of tobacco. Therefore, the findings of the current study can not be

interpreted as simply to claim that each two supplements would provide the same level of protection against tobacco products in general. In addition, further research should look into the effects of higher doses of quercetin or luteolin , and whether these effects might become prooxidant, as suggested by [42]. Future research should be able to elucidate the exact molecular mechanisms of quercetin or luteolin supplements protection against nicotine or other alkaloids and drugs.

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