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Flavone improves liver damage in nicotine-exposed rats *via* the Nrf2/HO-1 pathway

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ABSTRACT

Objective: To assess the hepatoprotective effects of flavone on nicotine-induced liver damage.

Methods: Thirty-six rats were allocated into six groups: the control group, the nicotine group, the flavone alone groups (10 and 25 mg/kg/body weight), and the nicotine groups treated with flavone (10 and 25 mg/kg/body weight). Liver function, oxidative stress, Nrf2 pathway (HO-1, Nrf2, and Keap-1), and inflammatory markers (IL-17, TNF- α , and NF- κ B) were evaluated. Additionally, a histopathological examination of liver tissues was performed.

Results: Nicotine increased liver damage, inflammation, and oxidative stress. However, flavone suppressed nicotine-induced liver enzymes, oxidative stress, and inflammation, as manifested by increased antioxidants and decreased malondialdehyde level, liver enzymatic activities, and inflammatory markers. Flavone (10 and 25 mg/kg/body weight) also reduced the level of Keap-1 and increased HO-1 and Nrf2 levels in the liver of nicotine-exposed rats.

Conclusions: Flavone has hepatoprotective properties and may slow the progression of liver injury by reducing oxidative stress, liver enzymes, and inflammation possibly *via* the Nrf2 pathway.

KEYWORDS: Flavone; Nicotine; Liver; Nrf2; HO-1; NF- κ B

1. Introduction

Nicotine, a highly toxic organic compound comprising nitrogen and alkaloid, primarily present in tobacco, is accountable for a portion of the adverse effects associated with smoking even though cigarettes contain nearly 4000 constituents[1]. Numerous cellular processes are influenced by nicotine, including gene expression. Nicotine exposure induces oxidative tissue damage in mice, frequently leading to reduced glutathione (GSH) levels and the functionality of oxygen-free radical scavengers, including superoxide dismutase (SOD)

and catalase[2]. Nevertheless, nicotine is recognized as its primary site of biotransformation in the liver, and it is worth noting that nicotine can cause various detrimental physiological consequences in the liver. Nicotine is swiftly metabolized in the liver after being absorbed through the lungs during smoking; this metabolism results in three significant detrimental impacts on the liver: immunological, oncogenic, and toxic (direct or indirect). Thus, the ingestion of cigarettes induces hepatotoxicity and damages liver cells in rats. Oxidative stress and compromised antioxidant defenses are key factors that contribute to the mediating effects of nicotine toxicity and the alterations associated with tobacco consumption. Nicotine exposure led to a reduction in cellular defenses and an increase in lipid peroxidation[3].

Many indicators suggest that cellular dysfunction can result from excessive reactive oxygen species (ROS) production that surpasses the antioxidative capacity of cells, thereby causing damage to cellular components, including DNA, protein, and lipids; however,

Significance

Besides the lung, cigarette smoking can negatively affect the liver, increasing the risk of liver diseases and cancer. In this study, flavone alleviated liver injury associated with nicotine by attenuating the inflammatory response and modulating the Nrf2/HO-1 signaling.

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tissues exposed to cigarette smoke extract exhibit a variety of detrimental effects linked to oxidative stress, including endothelial cell damage, cardiac hypertrophy, and pulmonary fibrosis. ROS are eliminated by nonenzymatic antioxidants GSH and a variety of enzymatic antioxidants, including catalase, SOD, and glutathione peroxidase (GPx)[4].

Nuclear factor erythroid 2-related factor 2 (Nrf2), as a redox-sensitive transcription factor, controls the transcriptional activation of antioxidative genes. However, Kelch-like-ECH associated protein 1 (Keap-1), a natural inhibitor of Nrf2, is bound to it in the cytoplasm under physiological conditions; in addition, Nrf2 dissociates from Keap-1 and translocates into the nucleus in response to oxidative or xenobiotic stimuli. Therefore, it binds to the antioxidant response element and activates the transcription of antioxidative genes, which encode proteins including heme oxygenase-1 (HO-1), SOD, catalase, and GPx[5]. In contrast, chronic cigarette smoke extract-induced pulmonary hypertension, alveolar destruction, alveolar cell apoptosis, and lung oxidative stress response in mice could be mitigated through Nrf2 activation[6]. Furthermore, an increasing body of evidence indicates that Nrf2 exerts protective effects on a multitude of organs and tissues, such as the liver[7]. Despite the protective role of Nrf2, its potential to shield the liver from oxidative injury induced by nicotine has not been the subject of any prior research.

Flavonoids are essential components in the well-being maintenance of both healthy and ailing individuals. Phytochemicals containing phenyl chromanone backbones (C6–C3–C6) with hydroxyl substitutions comprise nearly 5000 known flavonoids in various plants. The flavonoids mentioned above are categorized as anthocyanins, flavonols, flavones, flavanones, and isoflavones. Furthermore, antioxidant, radical scavenging, anti-inflammatory, anti-viral, anti-cancer, and anti-mutagenic properties are a few of the unique biological activities each flavonoid demonstrates *in vitro* and *in vivo* in mammalian systems[8]. However, color and fragrance in flowers are attributed to flavonoids, which are secondary metabolites of plants. Flavonoids inhibit angiogenesis, oxidative stress, and apoptosis by interfering with multiple signal transduction pathways implicated in liver diseases[9]. The inhibition of oxidative stress and related downstream responses, such as inflammatory diseases, is one of the significant underlying mechanisms of action of dietary flavonoids and related polyphenols. Flavonoids scavenge free radicals and their subsequent damage by producing relatively stable phenoxy radicals and metal chelation[10]. Furthermore, flavonoids interact with numerous gene products to obstruct their specific actions, thereby directly modulating a restricted response. For kinase inhibition, the interaction may affect numerous downstream pathways[11].

Additionally, flavone is an organic molecule with the formula $C_6H_4OC_3H(Ph)O$. Flavone is a chromone derivative containing a

phenyl (Ph) substituent next to the ether group. Recent research has shown that the AhR activity of flavones is contingent upon the compound, response, and cell context[12].

Recent research has demonstrated that flavone may have protective antioxidant properties through its ability to increase the activities of SOD, GPx, and catalase while decreasing lipid peroxidation. Furthermore, flavone demonstrated anti-apoptotic properties through upregulating B cell lymphoma-2 (Bcl-2) and downregulating caspase-3 and Bcl-2-associated protein X (Bax). Moreover, this compound reduced the expression levels of cluster of differentiation 31 (CD31) and transforming growth factor beta-1 (TGF- β 1)[13]. The existing research on flavone effects has not yet examined its potential as a protective agent against nicotine. Therefore, the purpose of this investigation was to assess the hepatoprotective effects of flavone against nicotine-induced hepatic injury in rats by determining liver function, oxidative stress, the Nrf2 pathway, and inflammatory markers.

2. Materials and methods

2.1. Compounds

All chemicals utilized in this investigation were purchased from Sigma-Aldrich® (St. Louis, Missouri, United States). Additionally, flavone was purchased for StarChem industrial chemicals (Egypt).

2.2. Animals

Thirty-six 12-month-old male Wistar rats weighing (240±10) g were obtained from the Egyptian National Authority for Drug Research and Control in Cairo, Egypt. During the experiments, all rats had free access to water and food. They were housed under comparable laboratory circumstances, including a 12-hour light/dark cycle, (50±15)% moisture, and (22±2) °C temperature.

2.3. Determination of LD₅₀ of flavone

Gradient concentrations from flavone (100 to 400 mg/kg) were administered orally to male Wistar rats to evaluate the median LD₅₀, which causes the death of 50% (LD₅₀) according to the study of Bass *et al.*[14]. Mortality after 24 h was monitored and LD₅₀ was calculated as follows:

$\text{Log LD}_{50} = \text{Log LD next below 50\%} + (\text{Log increasing factor} \times \text{proportionate distance})$

$\text{Proportionate distance} = (50\% - \text{mortality next below 50\%}) / (\text{mortality above 50\%} - \text{mortality next below 50\%})$

It was found that the LD₅₀ for flavone was 247.2 mg/kg body weight. Therefore, 1/10 and 1/20 of the calculated LD₅₀ dose were used.

2.4. Groups and treatment

The rats were assigned randomly into 6 groups: control group (rats received saline), flavone 10 group (rats orally received 10 mg/kg/body weight flavone five times/week for 2 weeks), flavone 25 group (rats orally received 25 mg/kg/body weight flavone five times/week for 2 weeks), nicotine group (rats received for 2 weeks 1 mg/kg/body weight nicotine intraperitoneally), nicotine-flavone 10 (rats were intraperitoneally given 1 mg/kg/body weight nicotine and then orally received for 2 weeks 10 mg/kg/body weight flavone five times/week), and nicotine-flavone 25 (rats were intraperitoneally administered with 1 mg/kg/body weight nicotine and then received for 2 weeks 25 mg/kg/body weight flavone five times/week). Subsequently, all rats were sacrificed with urethane anesthesia. Blood was collected through a cardiac needle and then left to clot to obtain serum. Additionally, liver tissues were promptly separated, rinsed with 0.9% saline, and then preserved at -80°C for subsequent examinations.

2.5. Real-time quantitative PCR (RT-qPCR)

Liver RNA was extracted employing the TRIzol (Life Technologies, USA) reagent. The total RNA concentration and quality (A260/A280 ratio) were analyzed using Nanodrop One (Thermo, Fisher Scientific, Inc., USA). The extracted RNA was reverse transcribed into cDNA using oligo dT primers and reverse transcriptase. RT-qPCR was performed in the 10 μL reaction system of SYBR Green qPCR Master Mix (Applied Biosystems TM). Subsequently, the reaction reagents were inserted, the reaction conditions were adjusted, and PCR was employed to amplify the gene. The relative expression of the target gene was normalized to β -actin using the $2^{-\Delta\Delta\text{Ct}}$ method at the end of the reaction[15].

The primer sequences were as follows: interleukin-17 (*IL-17*)[16]: forward: 5'-ACAGTGAAGGCAGCGGTACT-3', reverse: 5'-GCTCAGAGTCCAGGGTGAAG-3'; tumour necrosis factor- α (*TNF- α*)[17]: forward: 5'-TCCCAGAAAAGCAAGCAACC-3', reverse: 5'-TAGACAGAAGAGCGTGGTGG-3'; *NF- κ B*[18]: forward: 5'-CGTGAGGCTGTTTGTTTGA-3', reverse: 5'-TCTGCCCTCCTGACTCTACT-3'; β -actin[19]: forward: 5'-TCTTCCAGCCTTCCTTCCTG-3', reverse: 5'-CAATGCCTGGGTACATGGTG-3'.

2.6. Oxidative stress markers

The quantification of GPx was performed utilizing commercial kits supplied by BioDiagnostic, Giza, Egypt, in accordance with the method outlined in the brochure and as described by Paglia and Valentine[20]. Additionally, the quantification of malondialdehyde

(MDA) in the supernatants of tissue homogenate was performed utilizing a Bio-diagnostic reagent[21] in accordance with a colorimetric method. The levels of reduced GSH[22] were quantified in the liver homogenate supernatant using Bio-diagnostic reagents (Giza, Egypt) in accordance with the manufacturer's instructions.

2.7. Liver function test

The activities of liver alanine transaminase (ALT), aspartate transaminase (AST)[23], and alkaline phosphatase (ALP)[24] were determined in accordance with the protocols previously described using commercial kits obtained from Bio-diagnostic, Egypt. The activity of gamma-glutamyl transferase (GGT) was determined according to the method of Szasz[25].

2.8. Nrf2 pathway determination

HO-1[26], Nrf2[27], and Keap-1[28] levels were quantified using a commercial ELISA kit (MyBiosource Inc., San Diego, California, USA).

2.9. Histopathological examination of liver tissues

Liver tissue specimens were fixed in 10% formol saline, trimmed off, washed, and dehydrated in ascending grades of alcohol. The dehydrated specimens were cleared in xylene, embedded in paraffin blocks, and sectioned at 4-6 μm thickness. The obtained tissue sections were deparaffinized using xylol and stained using hematoxylin and eosin (H&E) for histopathological examination through the electric light microscope (Olympus xc30. Tokyo. Japan)[29]. The frequency and severity of lesions in the liver were assessed semi-quantitatively as previously reported[30] using a scale where grade 0: no apparent injury; grade I: swelling of hepatocytes; grade II: ballooning of hepatocytes; grade III: lipid droplets in hepatocytes and grade IV: necrosis of hepatocytes.

2.10. Statistical analysis

All values are displayed as mean $\pm$ SEM. SPSS software version 20 was utilized for one-way ANOVA accompanied by *post-hoc* multiple comparisons (LSD test) to determine the significant difference. $P < 0.05$ indicates statistical significance[31].

2.11. Ethical statement

This study was approved by the Institutional Animal Care and Use Committee Research Ethic Board of Benha University, Faculty of Veterinary Medicine (BUFVTM12-11-22).

3. Results

3.1. Flavone reduces nicotine-induced liver inflammation

Nicotine induced liver inflammation by significantly increasing the mRNA expressions of *IL-17*, *TNF- α* , and *NF- κ B* in rats compared with the normal control group ($P<0.01$). Flavone treatment at both doses significantly alleviated liver inflammation by significantly decreasing nicotine-induced upregulated mRNA expressions of *IL-17*, *TNF- α* , and *NF- κ B* ($P<0.01$). Meanwhile, flavone therapy in normal rats did not induce significant changes in inflammatory markers ($P>0.05$) (Figure 1).

3.2. Flavone ameliorates hepatic oxidative stress

Furthermore, nicotine evoked hepatic oxidative stress in rats.

Notably, the GSH level and GPx activity were remarkably decreased in the nicotine group compared to the normal control group ($P<0.01$), whereas the MDA level was significantly increased. In contrast, flavone treatment decreased hepatic oxidative stress by significantly ameliorating the MDA levels and increasing the GSH level and GPx activity in nicotine-exposed rats ($P<0.01$) (Figure 2). Meanwhile, flavone in normal rats did not induce significant changes in hepatic oxidative parameters (Figure 2).

3.3. Flavone improves nicotine-induced hepatic dysfunction

Nicotine triggered liver injury, as evidenced by markedly increased serum enzymatic activities, including AST, ALT, ALP, and GGT ($P<0.01$) (Figure 3). However, treating nicotine-exposed rats with flavone improved liver damage by significantly decreasing enzymatic activities, including AST, ALT, ALP, and GGT ($P<0.01$).

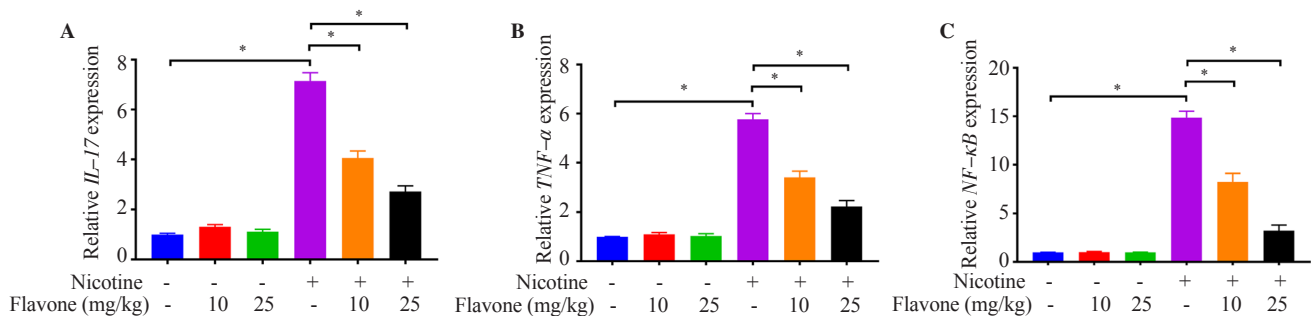


Figure 1. Effect of flavone on nicotine-induced liver inflammation. (A) *IL-17*, (B) *TNF- α* and (C) *NF- κ B* mRNA transcript levels were determined by RT-qPCR. The data are expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by LSD test. * $P<0.01$.

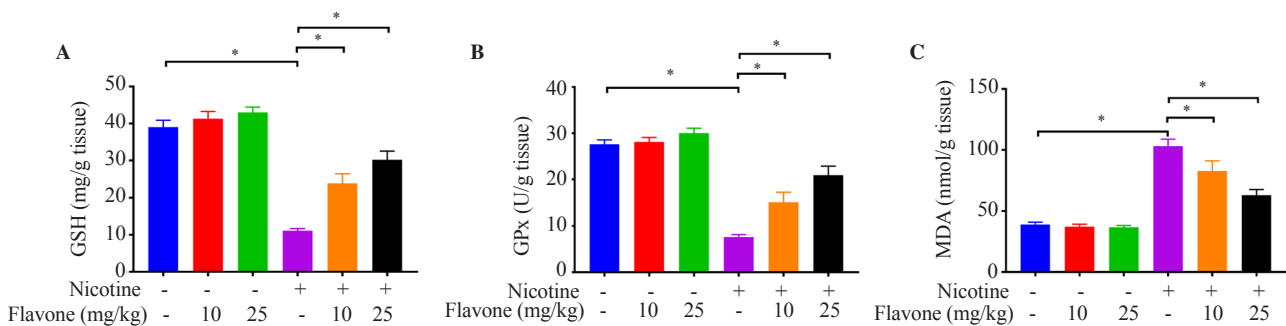


Figure 2. Effect of flavone on nicotine-induced oxidative stress. The data are expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by LSD test. * $P<0.01$. GSH: glutathione; GPx: glutathione peroxidase; MDA: malondialdehyde.

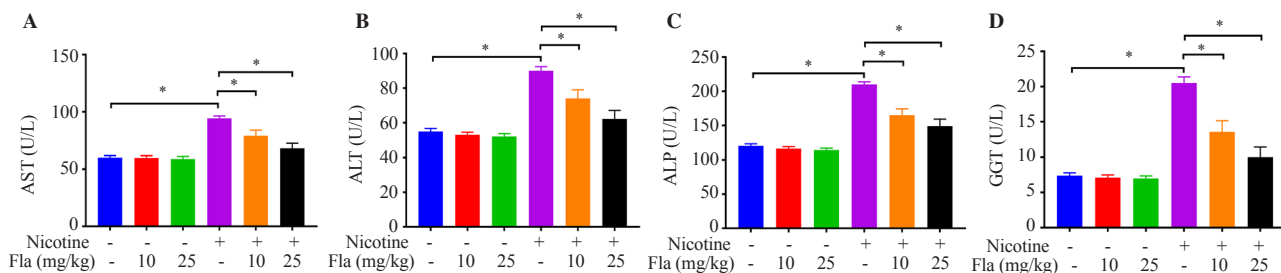


Figure 3. Effect of flavone on nicotine-induced hepatic dysfunction. (A) Aspartate transaminase (AST), (B) alanine transaminase (ALT), (C) alkaline phosphatase (ALP), (D) gamma-glutamyl transferase (GGT). The data are expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by LSD test. * $P<0.01$. Fla: flavone.

(Figure 3). Meanwhile, treatment with flavone alone did not cause significant changes in enzymatic activities ($P>0.05$) (Figure 3).

3.4. Flavone improves liver damage, inflammation, and oxidative stress via the Nrf2 pathway

Nicotine significantly decreased Nrf2 and HO-1 levels while raising Keap-1 level in the liver of rats ($P<0.01$). However, flavone treatment at both doses reversed nicotine's effect by increasing the expression of Nrf2 and HO-1 levels and reducing Keap-1 level ($P<0.01$) (Figure 4).

3.5. Flavone improves nicotine-induced histological damage in the liver

The liver tissue section of the normal control rats showed the

normal histological structure of hepatic lobules and the organization of hepatic cords with a prominent central hepatic vein. Polygonal hepatic cells were joined in anastomosing plates, with borders that faced either the sinusoids or adjacent hepatocytes (grade 0) (Figure 5A). On the other hand, rats exposed to nicotine showed disorganization of hepatic cords and dilatation of hepatic sinusoids. A few of intracellular fat droplets were noticed (grade III) (Figure 5B).

Liver tissue sections of rats treated with flavone 10 mg/kg or 25 mg/kg alone showed the normal histological structure of hepatic lobules similar to the normal control group without any significant pathological alterations. Organization of hepatic cords with polygonal hepatic cells joined to one another that face either the sinusoids or adjacent hepatocytes (grade 0) was observed (Figure 5C-D).

Liver tissue sections of rats exposed to nicotine and flavone 10 mg/

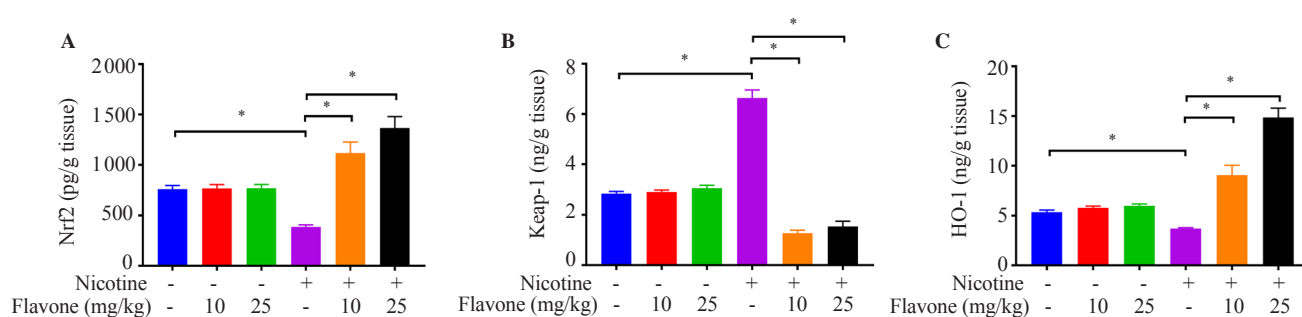


Figure 4. Effect of flavone on the Nrf2 pathway in the liver of nicotine-exposed rats. (A) Nrf2, (B) Keap-1 and (C) HO-1 levels were quantified by ELISA method. The data are expressed as mean $\pm$ SEM ($n = 6$) and analyzed by one-way ANOVA followed by LSD test. * $P<0.01$.

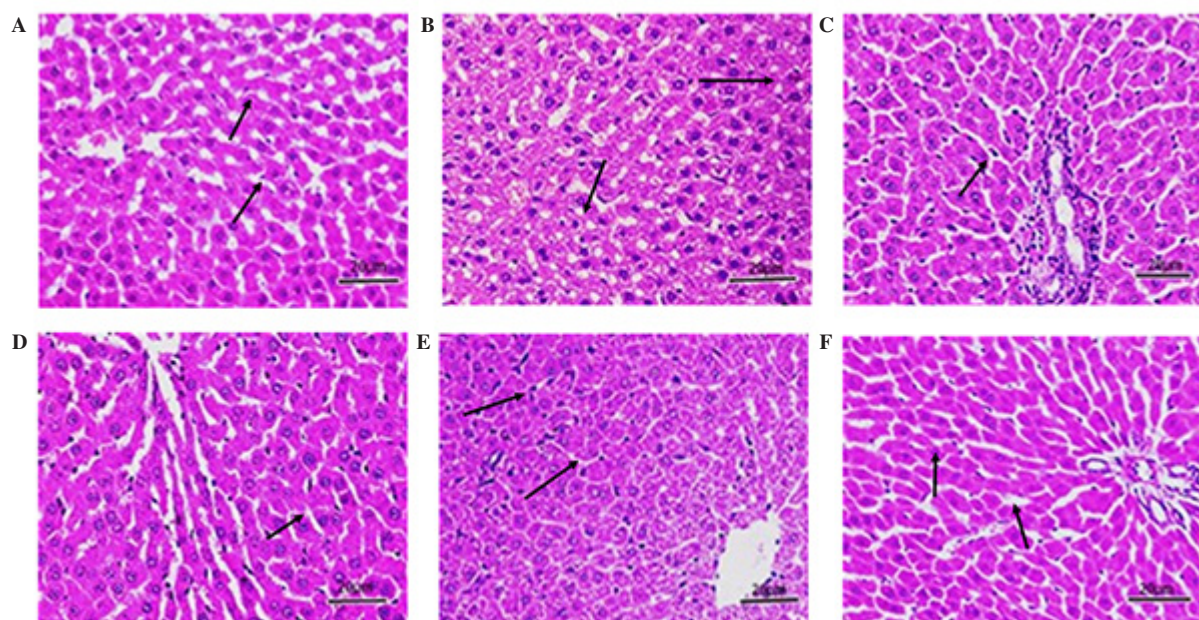


Figure 5. Effect of flavone on nicotine-induced liver histological changes (H&E $\times 200$). (A) The normal control group has a normal histological structure of hepatic lobules, which consists of strands of polygonal cells (arrows). (B) The nicotine group shows a few number of intracellular fat droplets (arrows). (C-D) The flavone alone groups at 10 and 25 mg/kg demonstrate a normal histological structure of hepatic lobules (arrows). (E) The nicotine group treated with 10 mg/kg flavone shows ballooning degeneration of hepatocytes and narrowing of hepatic sinusoids with hyperplasia of Kupffer cells (arrows). (F) The nicotine group treated with 25 mg/kg flavone exhibits a mild swelling of hepatocytes (arrows).

kg revealed ballooning degeneration of hepatocytes and narrowing of hepatic sinusoids with hyperplasia of Kupffer cells (grade II) (Figure 5E). Conversely, rats exposed to nicotine and flavone 25 mg/kg revealed mild swelling of hepatocytes and granularity of its cytoplasm with centrally situated vesiculated nuclei with peripheral nuclear chromatin condensation. Dilatation of hepatic sinusoids with hyperplasia of Kupffer cells was also noticed (grade I) (Figure 5F).

4. Discussion

Egypt is among the nations most significantly impacted by the dangers of smoking; therefore, the prevention of tobacco use in Egypt is currently a formidable challenge. A National Health Survey in Egypt (2018) found that tobacco use is prevalent among 22.8% of the Egyptian population. As per the Centers for Disease Control and Prevention[32], smoking is the primary contributor to preventable illnesses and fatalities in both Egypt and the United States, resulting in over 34000 and 480000 deaths annually, respectively.

Cigarette smoking is prevalent in developing nations and the primary noxious constituent of cigarette smoke is nicotine[33]. It appears that the liver is the most appropriate organ to provide an accurate assessment of the degree of toxicity induced by nicotine in the blood or tobacco inhalation. Additionally, the liver is particularly susceptible to nicotine because it is where the majority of nicotine's metabolism and biotransformation occur[34]. Developing therapeutic tools to prevent and mitigate the adverse effects of cigarette smoking is a significant public health objective. Hence, the primary objective of the present investigation was to ascertain whether flavone possesses prospective protective properties against nicotine-induced

hepatic injury in rats.

In the current study, nicotine administration for two weeks induced liver injury as evidenced by increased levels of liver enzymes in serum. Nicotine evoked oxidative stress in liver tissues. Furthermore, nicotine decreased Nrf2 and HO-1 protein levels while increasing TNF- α , NF- κ B, IL-17, and Keap-1 levels in liver tissues. Treatment with flavone (10 or 25 mg/kg) attenuated nicotine-induced liver damage, as indicated by attenuated oxidative stress and inflammation. These results suggest that flavone might have potential effects against nicotine-induced liver damage by shifting the balance to favor the production of antioxidants rather than the production of pro-oxidants and proinflammatory cytokines.

In the present investigation, nicotine administration resulted in a substantial elevation of liver enzymes compared to the normal control group. Flavone administration brought these enzymes closer to their baseline levels. Such enzymes may be released into the bloodstream due to necrosis or cell membrane damage. Moreover, liver performance correlates with these enzymes' serum concentration. Nicotine appears to induce hepatic cell membrane disintegration, which releases cytosomal enzymes from hepatocytes and ultimately elevates their concentration in the bloodstream. The reduction in enzyme levels observed in the groups that were administered with varying concentrations of flavone suggests that flavone exerts a protective effect against nicotine-induced toxicity. The observed outcomes might be ascribed to flavone's antioxidant properties and capacity to mitigate oxidative stress. These findings are consistent with the results of a previous study, in which flavonoids have a beneficial effect against liver damage[35].

Nicotine metabolism induces free radicals that catalyze lipid peroxidation and DNA and membrane protein reactions,

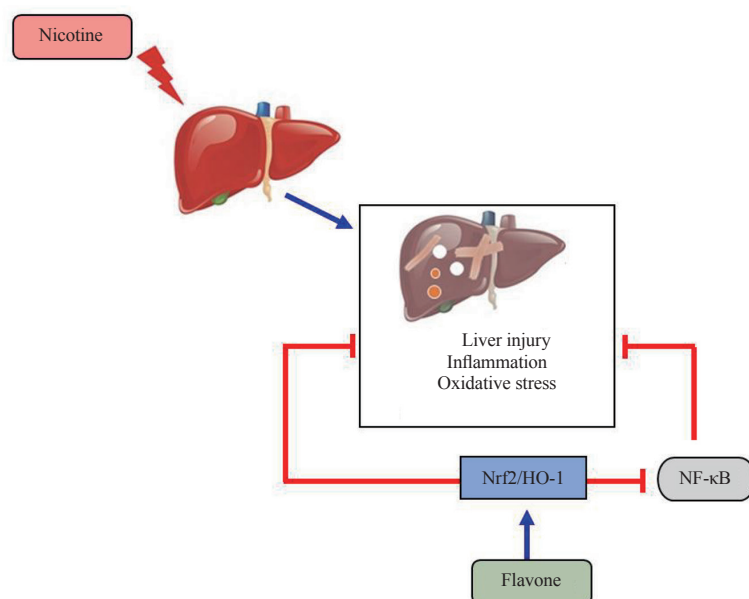


Figure 6. Flavone inhibits nicotine-induced liver damage, inflammation, and oxidative stress *via* activating Nrf2/HO-1 and inhibiting the NF- κ B pathways.

consequently inducing cellular damage. However, it could be ascribed to a reduction in the concentration of GSH and its peroxidase activity[36]. Significant evidence suggests that nicotine induces oxidative stress due to ROS, lipid peroxidation, and DNA damage, which antioxidants can effectively counteract[37]. Additionally, nicotine-induced oxidative stress develops when an abundance of free radicals and/or a deficiency in antioxidant defense occurs, leading to chemical modifications of biomolecules that impact their structure and function[36]. Cumulative evidence suggests that nicotine exposure induces cellular injury, which is linked to an imbalance in the oxidant-antioxidant system of the cell.

Surprisingly, flavone, an antioxidant compound, can impede nicotine metabolism, resulting in a subsequent decrease in free radical production. Recent research has demonstrated that flavonoids may have protective antioxidant properties through their ability to increase the activities of SOD, GPx, and catalase while decreasing lipid peroxidation.

Additionally, proinflammatory cytokines may contribute to hepatic inflammation, Kupffer cell activation, and hepatocyte damage[38]. The administration of nicotine in this study resulted in a substantial elevation of IL-17, TNF- α , and NF- κ B compared to the normal control group. Flavone can mitigate the detrimental effects of liver toxicity by impeding the secretion of proinflammatory cytokines, which are factors in the induction of hepatic necrosis. Consistent with the findings of the present study, previous reports revealed that nicotine predominantly induces tissue inflammation through the upregulation of TNF- α and NF- κ B expression[39]. Flavone administration brought these inflammatory markers closer to their baseline level, suggesting that it can protect against liver injury *via* anti-inflammatory effects that reduce the formation of ROS and the downregulation of many inflammatory mediators[40].

NF- κ B, a crucial nuclear transcription factor, induces the expression of numerous genes implicated in inflammation, including TNF- α . Previous research demonstrated that nicotine elevated NF- κ B protein concentrations in the liver, lungs, and kidneys[41]. Nicotine-associated ROS induce phosphorylation of inhibitory protein kappa B-alpha (I κ B)- α by inhibitory kappa B kinase (IKK)- β , followed by ubiquitination and degradation[42]. NF- κ B p65 dissociates from I κ B- α and enters the nucleus, stimulating the transcription of various genes, including TNF- α and iNOS, stimulating further ROS production[41]. Additionally, nicotine is known to stimulate the α 7 nicotinic acetylcholine receptor on macrophages, which triggers the secretion of proinflammatory mediators and neutrophil activation[41]. This results in the production of inflammatory cytokines and free radicals, including TNF- α , which are implicated in systemic inflammation and tissue damage. The current study showed that nicotine increased protein levels of NF- κ B in liver tissues. Flavone administration for 2 weeks along with nicotine reduced NF- κ B protein levels in liver tissue compared to nicotine-exposed rats,

suggesting its anti-inflammatory effect in the nicotine murine model[40].

The Nrf2/HO-1 signaling pathway is a central antioxidant activity regulator. Nrf2 potentially plays a pivotal role in the cellular defense mechanisms through which hepatocytes resist oxidative stress[43]. Consistent with these results, the present investigation demonstrated that nicotine decreased Nrf2 levels in the liver tissue compared with the normal control group. Increasing antioxidant enzyme activity and upregulating Nrf2 protein levels can mitigate nicotine-induced oxidative stress[44]. The current investigation found that the administration of flavone mitigated the decrease in Nrf2 levels induced by nicotine. This finding implies that the upregulation of Nrf2 could mediate the antioxidant effect of flavone.

In order to elucidate the mechanism by which flavone exerts its antioxidative effects, we examined the impact of flavone on the expression of Nrf2 in the liver of rats. While growing evidence suggests that Nrf2 plays a significant role in cellular defense mechanisms against oxidative stress, its impact on antioxidative genes has been extensively documented[45], but the effects of flavone on the Nrf2 pathway have never been described in the liver. Nrf2/Keap-1 is involved in crosstalk with the NF- κ B signaling pathway. Nrf2/Keap-1 controls NF- κ B activation *via* the reduction of I κ B α phosphorylation, promoting NF- κ B degradation[46]. Additionally, previous studies have documented that Nrf2 knockout mice exhibited elevated NF- κ B activity compared to their wild-type counterparts[47].

However, our study has some limitations. The duration of the study was short and needed to be increased to explore whether flavone has any side effects or not. Additionally, only liver tissues were evaluated; however, nicotine hurts various body organs. Further investigations and analyses are also needed to explore the exact mechanisms underlying the hepatoprotective effects of flavone.

In conclusion, the obtained results revealed that flavone ameliorated liver injury, as evidenced by improved liver function and structure and reduced inflammatory response. Moreover, it attenuated the impaired redox status, most likely *via* boosting the antioxidant levels (GSH and GPx) concomitant with the activation of the Nrf2 pathway (Figure 6).

Conflict of interest statement

The authors declare there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

NAE conducted all experimentation. FSMM and ESAA conceived the study, provided data, experimental design, and helped write the manuscript. AH conceived the study and directed it, and wrote the manuscript. OARAZ conceived the study and supervised the work. All authors agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

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