Ameliorative Effects of Fenugreek Seeds and Curcumin against Hematoxicity Induced by Nicotine in Male Albino Rats

Azab Elsayed Azab¹, Mohamed Omar Albasha², Manal Abuelkasem Elnaif ³

¹Physiology Department, Faculty of Medicine, Sabratha University, Libya, ²Zoology Department, Faculty of Science, Alejalat, Zawia University, Libya, ³Zoology Department, Faculty of Science, Zawia University, Libya

ABSTRACT

The present study aimed to investigate the ameliorative effects of fenugreek seeds and curcumin against hematoxicity induced by nicotine in male albino rats. 30 male F-344/NHsd Fischer rats, weighing from 180 to 200g were used in the present study. The animals were divided into five groups (6 rats for each); Group I (control group), Group II (nicotine treated group), Group III (nicotine/fenugreek seeds co-administered), Group IV (nicotine/curcumin co-administered), and Group V (nicotine/curcumin& fenugreek seeds co-administered). At the end of the experimentation and 24 hours after the last dose, All animals were anaesthetized with ether and blood samples were collected by heart puncture. The samples were collected in clean dry tubes containing the anticoagulant substance EDTA and used for the hematological studies. The results showed that the animals treated with nicotine for 4 weeks showed a significant decrease in RBCs count, hemoglobin concentration, hematocrit value, MCH, MCHC, and platelets count, and increased MCV and WBCs count as compared to the control group. Co-administration of nicotine with fenugreek and/or curcumin caused improvement in all hematological when compared with nicotine group. It can be concluded that nicotine had a strong effect on the hematological parameters. The ingestion of fenugreek and/or curcumin prevent the hematoxicity induced by nicotine. The current study suggests that fenugreek and curcumin may be useful in combating free radical-induced hematotoxicity induced by nicotine.

Key words: Nicotine, Hematotoxicity, Fenugreek, Curcumin, Co-administration, Male albino rats.

INTRODUCTION

Cigarette smoking and the use of other tobacco products became an important cause of increased mortality and morbidity in developed countries,¹ because it increases the risk of heart disease, diabetes, lung cancer, respiratory disorders, and other illnesses.²

Nicotine is one of hundreds of substances contained in cigarette smoke.¹ It is a highly toxic organic compound containing nitrogen and alkaloid which is mostly found in tobacco,³ and responsible for its addiction.⁴

Nicotine may be induce production of free radicals and consequently oxidative stress.⁵ People who smoke and also who are exposed to cigarette smoke indirectly by breathing the air in the same environment are exposed to nicotine induced oxidative stress.⁶⁷ Oxidative stress would result in increased free radical injury in the tissue leading to extensive tissue damage with subsequent derangement of...
cell physiology. As a consequence, these radicals interact with cell components such as lipids, proteins, DNA, RNA, carbohydrates and enzymes. So, that smoking has an effect on the various metabolic and biological processes in the body.

Nicotine can easily pass through the cell membrane and react to tubulin protein present in the cytoplasm of multiplying cells and cause cell division disorder. It increases the risk of coronary artery disease and promotes tumor growth as well as atherosclerosis formation. Also, nicotine consumption can decrease fertility drive in males through inducing oxidative stress and DNA damage.

The body is engaged in a constant battle against damaging chemicals called free radicals or pro-oxidants to counter the harmful effects of free radicals, the body manufactures antioxidants to chemically neutralize them. However, the natural antioxidant system may not always be equal to the task. Sources of free radicals, such as cigarette smoke may overwhelm this defense mechanism.

Natural antioxidants strengthen the endogenous antioxidants defenses and restore the optimal balance by neutralizing reactive species. Curcumin as one of the naturally occurring dietary substances has been used since ancient times for promoting human health. Curcumin is a major yellow pigments in rhizomes of Curcuma longa which is used widely as a spice and coloring agent in several foods. It represents a class of anti-inflammatory and anti-oxidant reported to be a potent inhibitor of reactive oxygen species formation.

Fenugreek (Trigonella foenumgraecum) is an annual herb belonging to Legume family; it is widely grown in India, Egypt, and Middle Eastern countries. It used both in medicine and with food as spice show antioxidant effect through their used in diabetes mellitus due to the presence of different active constituents such as flavonoids, alkaloids, vitamins and amino acids. The yellowish seeds contain compounds with interesting proprieties which explain their use in various ways including medicine, nutrition, beverages, fragrances, cosmetics, smoking and for other industrial purposes. In fact, toasted and ground fenugreek seed is an essential ingredient of curry powders and is often mixed with breadstuffs.

Plant seeds and herbs are used for treatments of diseases in the folk medicine. Their use was increased in many fields due to their safetyness and its low side effects as compared with chemical drugs. Antioxidant potential of curcumin and fenugreek seeds in the amelioration of nicotine induced oxidative stress need thorough investigation because these natural antioxidants are components of many edible substances and has the potential for safe future use by humans. The evidence reporting the protective effect of curcumin and fenugreek seeds against nicotine induced haemato-toxicity are hardly found.

**OBJECTIVES**

The present study aimed to Evaluate the protective effects of fenugreek seeds, and curcumin on hematotoxicity induced by nicotine in male albino rats

**MATERIALS AND METHODS**

**Experimental Animal**

Animals which were used in this study were 30 male F-344/NHsd Fischer rats, weighing from 180 to 200g. Animals purchased from Animal Welfare House of Libyan National Medical Research Centre, Zawia, Libya. Rats were kept under standard veterinary hygienic conditions for cleanliness and health care and normal conditions through the whole experimental periods. Rats were separated in plastic cages, 6 rats per cage, and left one week of acclimation, before commencing the experiment. The rats were kept in a room under standard conditions of ventilation, temperature (25±4°C), humidity (65 ± 5 %) with light/dark cycle. A standard rodent pellet consisting of a mixture of protein, fat, fiber, and ash were used to feed the rats. Food and water were supplied ad-libitum.

**Methods and Technique**

**The Drug**

Nicotine hydrogen tartrate salt (1-methyl-2-(3-pyridyl) pyrrolidine-bitartrate salt) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Nicotine is a colorless organic Liquid. It was dissolved in physiological saline (0.9% sodium chloride) and was injected subcutaneously daily with 0.8 mg, nicotine/kg body weight for 30 days.

Curcumin and Fenugreek Seeds

Curcumin was given in diet as 20 g/kg diet daily for 30 days. Fenugreek seeds were finely grounded and added to the experimental diets as 7.5 g/kg diet daily for 30 days.

**Experimental Design**

After one week of acclimation, the animals were randomized and divided into five groups (6 male albino rats for each) as follow:

- **Group I (control group):** This group included 6 animals that were injected subcutaneously with saline daily, provided with tape water and fed with normal diet for 30 days.
- **Group II (nicotine treated group):** Male rats were injected subcutaneous daily with 0.8 mg, nicotine/kg body weight for 30 days.
- **Group III (nicotine/fenugreek seeds co-administered):** The
animals were injected subcutaneous daily with 0.8 mg, nicotine/kg body weight concurrently with fenugreek seeds 7.5 g/kg diet daily for 30 days.

Group IV (nicotine/curcumin co-administered): The animals were injected subcutaneous daily with 0.8 mg, nicotine/kg body weight concurrently with curcumin 20 g/kg diet daily for 30 days.

Group V (nicotine/curcumin & fenugreek seeds co-administered): The animals were injected subcutaneous daily with 0.8 mg, nicotine/kg body weight concurrently with curcumin 20 g/kg diet and fenugreek seeds 7.5 g/kg diet daily for 30 days.

**Blood Sampling**

At the end of the experimentation and 24 hours after the last dose, All animals were anaesthetized with ether and blood samples were collected by heart puncture. The samples were collected in clean dry tube containing the anticoagulant substance EDTA (ethylene diamine tetra acetic acid) and used for the hematological studies.

**Determination of Haematological parameters**

Red blood cells count, haemoglobin concentration, hematocrit value, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cells count, differential count of leucocytes, and blood platelets count were determined using an automated haematology analyzer Sysmex (KX. 21) machine.

**Statistical Analysis**

Results were expressed as mean ± standard deviation. Data were analyzed by one way ANOVA. The difference between means ± SD was tested at P<0.05 using Duncan’s multiple range test. In all statistical tests, the probability level of P<0.05 was considered significant.

**RESULTS**

Effect of administration of nicotine, and co-administration of nicotine with fenugreek seeds, nicotine with curcumin and nicotine, fenugreek seeds, and curcumin on haematological parameters in male rats.

Haematological parameters of the different groups are shown in table 1. Male rats that received intraperitoneal injection of nicotine only (0.8 mg/kg body weight/day) for 30 consecutive days had significantly (P<0.01), decreased RBCs count, hemoglobin concentration, hematocrit value, MCH, MCHC, and platelets count, and increased MCV and WBCs count as compared to the control group.

The results of the study showed that the male rats injected subcutaneously daily with 0.8 mg, nicotine/kg body weight concurrently with fenugreek seeds 7.5 g/kg diet daily for 30 consecutive days resulted in a significant (P<0.01) decrease in RBCs count, MCH, MCHC, and platelets count, and at (P<0.05) in hemoglobin concentration, and hematocrit value, and a significant (P<0.01) increased in MCV and WBCs count as compared to the control group (Table.1). Conversely, co-administration of fenugreek seeds with nicotine significantly (P<0.01) improved all haematological parameters when compared with nicotine group (Table.1).

Co-administration of 0.8 mg, nicotine/kg body weight subcutaneously with curcumin 20 g/kg diet daily for 30 consecutive days caused a significant (P<0.01) decrease in RBCs count, hemoglobin concentration, and hematocrit value, MCH, MCHC, and platelets count, and a significant (P<0.01) increased in MCV and WBCs count as compared to the control group. Conversely, co-administration of curcumin with nicotine significantly (P<0.01) improved all haematological parameters when compared with nicotine group (Table.1).

The animals injected subcutaneous daily with 0.8 mg, nicotine/kg body weight concurrently with curcumin 20 g/kg diet and fenugreek seeds 7.5 g/kg diet daily for 30 consecutive days were showed a significant (P<0.01) decrease in MCH, MCHC, and platelets count, and a significant (P<0.01) increased in MCV and WBCs count as compared to the control group (Table.1). Conversely, co-administration of fenugreek and curcumin with nicotine significantly (P<0.01) improved all haematological parameters when compared with nicotine group (Table.1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Nicotine</th>
<th>Nicotine+ Fenugreek</th>
<th>Nicotine+ Curcumin</th>
<th>Nicotine+ Fenugreek+ Curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>RBCs (x10^6/µL)</td>
<td>9.7 ± 0.2</td>
<td>7.7 ± 0.4&quot;</td>
<td>8.9 ± 0.1&quot;**</td>
<td>8.6 ± 0.1&quot;##</td>
<td>9.3 ± 0.1&quot;###</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>15.1 ± 0.2</td>
<td>11.9 ± 0.9&quot;</td>
<td>14.3 ± 0.2&quot;##</td>
<td>13.9 ± 0.2&quot;##</td>
<td>14.8 ± 0.1&quot;###</td>
</tr>
</tbody>
</table>

Table 1. Effect of administration of nicotine, and co-administration of nicotine with fenugreek seeds, nicotine with curcumin and nicotine, fenugreek seeds, and curcumin on haematological parameters in male rats.
### Discussion

In the current study, male rats that received intraperitoneal injection of nicotine only (0.8 mg/kg body weight /day) for 30 consecutive days had significantly (P<0.01), decreased RBCs count, hemoglobin concentration, hematocrit value, MCH, MCHC, and platelets count, and increased MCV and WBCs count as compared to the control group. These results are similar with the study of,[23] who reported that mice injected with 1 mg/kg body weight of nicotine daily for 6 weeks caused a significant (p<0.05) increase in hematocrit, mean corpuscular volume, and white blood cells, and a significant decrease in RBCs count, mean corpuscular hemoglobin, hemoglobin, and mean corpuscular hemoglobin concentration compared with control group. [24] recorded that smoking caused a significant increase in WBCs count and decrease in RBCs count. Also, nicotine causes many changes in blood cells as it simply diffuses into the cells.[25,26] Also, [27] reported that cigarette smoking caused a significant (p<0.05) decreased hemoglobin level.[28] reported that smoking caused a significant increase in MCV and a significant decrease in MCH and MCHC. The previous studies[23, 29, 30] showed that nicotine administration caused a decrease in proliferation of red blood cells and as a result the RBCs count decreases. Low erythrocytes count may lead to a number of physiological disorders.[23] Nicotine greatly suppresses the function of immune system and due to this reason the number of WBCs increased in the body to strengthen the immune system.[23,31]

Co-administration of fenugreek seeds with nicotine significantly (P<0.01) improved all hematological parameters, increase in RBCs count, MCH, MCHC, and platelets count, and at (P<0.05) in hemoglobin concentration, and hematocrit value, and a significant (P<0.01) decreased in MCV and WBCs count as compared to the nicotine treated group. These results run parallel to those reported by many previous studies.[32-34] The study of,[35] reported that treatment of rats with 10% ethanol in drinking water for 30 days caused a significant increase in RBCs count, Hct value, and Hb concentration, WBCs count, and lymphocytes percentage and a decrease in neutrophils percentage as compared to the control animals. Addition of 10% fenugreek flour in the diet of ethanol-intoxicated rats for 30 days showed a tendency to restore the control values.[36] reported that fenugreek oil was ameliorated the altered hematological parameters in diabetic rats through its antioxidant properties, that may be due to their content of polyphenolic flavonoids.[37, 38]

Also,[39] recorded that rats treated with 15 mg/ kg bw deltamethrin orally showed a significant decrease in RBCs and platelet counts, hemoglobin concentration, and hematocrit value and a significant increase in leucocytes count when compared with the control group. But, co-administration of rats with fenugreek oil contained diets (2.5% and 5%) and 15 mg/ kg bw deltamethrin orally resulted in a significant increase in RBCs and platelets counts, hemoglobin concentration and hematocrit value and a significant decrease in leucocytes count as compared with deltamethrin treated rats. Fenugreek oil kept the studied hematological parameters within normal ranges. Thus, including fenugreek oil in the diets of deltamethrin administrated rats prevented the oxidative stress induced by deltamethrin, which subsequently protects the immune and hemopiotic organs.[40] reported that fenugreek seeds influenced the hemoglobin and lymphocytes count, improving hematopoietic function.[41] demonstrated that Feeding of rats on a diet supplemented with fenugreek seeds at a concentration of 5% before and after 14 days of irradiation exposure significantly increased hemoglobin and lymphocytes percentage compared to the control group. Also, it was demonstrated the role of fenugreek seeds in protecting the spleen and increased lymphocyte, suggesting that fenugreek seeds might improve immunity.[42] reported that lactating female rabbits treated with fenugreek germinated and powdered seeds showed a significant increase in RBCs count, Hb concentration Hct, and MCH values. Administration of fenugreek-germinated seeds; oil or powdered seeds to lactating female rabbits were improved RBCs count, Hct, Hb, blood indices, and WBCs count.[43] reported that streptozotocin-induced diabetic rats caused a significant decrease in RBCs count, Hb concentration, MCV, Hct value, MCH, MCHC, and platelets count in diabetic rats. Hyperglycemia increases the production of free radicals and oxidative stress that in turn is a cause of cellular dysfunction. Dietary fenugreek seeds (100 g/kg) and onion (30g/kg) treatment of streptozotocin-induced diabetic rats, appeared to counter the deformity of erythrocytes partially in diabetic rats for 30 days showed a tendency to restore the control parameters. The study of,[44] reported that the administration of fenugreek seeds to streptozotocin-induced diabetic rats significantly increased RBCs count, Hb concentration, and Hct value, while decreased MCV and WBCs count.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Fenugreek 10%</th>
<th>Fenugreek 20%</th>
<th>Fenugreek 30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (10^6/μL)</td>
<td>6.5 ± 0.7</td>
<td>14.4 ± 1.2&quot;</td>
<td>10.7 ± 0.3&quot;</td>
<td>11.9 ± 0.6&quot;</td>
</tr>
<tr>
<td>WBCs (10^3/μL)</td>
<td>2021± 54</td>
<td>1282± 155&quot;</td>
<td>1840± 65&quot;</td>
<td>1605± 83&quot;</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>54.2 ± 1.2</td>
<td>43.5 ± 3.3&quot;</td>
<td>50.4 ± 0.3&quot;</td>
<td>48.2 ± 0.7&quot;</td>
</tr>
<tr>
<td>MCV (μL)</td>
<td>52.5 ± 1.5</td>
<td>60.9 ± 1.5&quot;</td>
<td>55.9 ± 0.5&quot;</td>
<td>57.4 ± 0.6&quot;</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>17.0 ± 0.4</td>
<td>14.9 ± 0.2&quot;</td>
<td>15.8 ± 0.1&quot;</td>
<td>15.3 ± 0.2&quot;</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>30.6 ± 0.8</td>
<td>26.3 ± 0.4&quot;</td>
<td>27.9 ± 0.4&quot;</td>
<td>27.1 ± 0.3&quot;</td>
</tr>
<tr>
<td>PLTs (10^9/μL)</td>
<td>51.5 ± 0.7</td>
<td>60.9 ± 1.5&quot;</td>
<td>54.7 ± 0.3&quot;</td>
<td>29.1 ± 0.1&quot;</td>
</tr>
</tbody>
</table>

*: Significant at (P<0.05) when compared with control group, **: Significant at (P<0.01) when compared with nicotine group.
rats by their antioxidant potential. Dietary fenugreek seeds and onion caused a decrease in glycated haemoglobin, and a nephro-protective probably mediated by stimulating erythropoietin which enhances rapid synthesis of RBCs as indicated by the improved level of MCH and MCHC in diabetes treated groups. reported that the combination treatment of rats with Glimepiride and a fenugreek aqueous extract in streptozotocin induced diabetic in male albino rats for eight weeks caused an improvement in RBC count, Ht value, Hb concentration, MCHC value, platelets count, and total WBCs count compared with the diabetic rats.

In addition, recorded that treatment of male rabbits with aluminum chloride were decreased red blood cell count, hemoglobin concentration, hematocrite, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume values, and a significant increase in WBCs count, differential count of leukocytes, and platelets count as compared with the control rabbits. Co-administration of fenugreek seeds powder to male rabbits with aluminum chloride resulted in a significant improvement in hematological parameters.

The improvement in hematological parameters caused by treatment with fenugreek may be due to the antioxidant activity of flavonoids present in fenugreek seeds, thereby elevating the antioxidant capacity of the blood, the antioxidant property of fenugreek inhibits lipid peroxidation of the erythrocytes, and the high iron content of fenugreek seed flour stimulated hemoglobin synthesis. Also, fenugreek seeds extract showed protective effects against hydrogen peroxide-induced oxidation by protecting the erythrocytes from hemolysis and lipid peroxidation due to the presence of flavonoids and polyphenols. Fenugreek seeds may be improving immunity because they play a role in protecting the spleen and increasing the lymphocytes. suggested that the administration of fenugreek powdered seeds were responsible for improvement of Immunological profile through increase phagocytic index, phagocytic capacity of macrophages, and humoral immunity.

Improvement in platelet count may be due to the inhibitory activity of certain constituents of fenugreek on platelet aggregation.

The current study showed that co-administration of 0.8 mg, nicotine/kg body weight subcutaneously with curcumin 20 g/kg diet daily for 30 consecutive days caused a significant decrease in RBCs count, hemoglobin concentration, and hematocrit value, MCH, MCHC, and platelets count, and a significant increased in MCV and WBCs count as compared to the control group. Conversely, co-administration of curcumin with nicotine significantly improved all haematological parameters when compared with nicotine group. These results run parallel to the results of who reported that mice exposed to gasoline vapor 2 hours/day for 3 weeks in inhalation chamber showed a reduction in bone marrow cellularity and slow rate of cells maturation. Apoptosis appeared in bone marrow cells by histopathological examination for biopsies. Also, reduction in blood cell counts was occurred, in RBCs, WBCs, platelets, and hemoglobin. Lymphocytes percentages in blood were depressed and neutrophils percentages were elevated in gasoline inhalation group. All these were improved and returned to the normal levels by providing mice with curcumin in the diet. Curcumin protected leukocytes from depression caused by gasoline. This effect of curcumin on hematopoeisis may be due to its strong inhibiting effect on myeloperoxidase activity which is the corner stone enzyme in benzene hematotoxicity.

The immunomodulatory functions of curcumin had appeared in the study of when WBCs count, circulatory antibody titer against sheep RBCs, the plaque forming cells in the spleen, significantly increased with curcumin administration to Balb/c mice. estimated that curcumin strongly inhibited myeloperoxidase activity in vitro. recorded that curcumin administration to tumor-eating mice decreased tumor cell number significantly in a dose-dependent manner. Furthermore, tumor induced depletion of immune cell number of the host, as was evidenced from the decrease in bone marrow progenitor as well as thymic and splenic mononuclear cell numbers was reinitiated by curcumin. Moreover, rather in tumor-bearing mice it inhibited hematopoietic toxicity, and activated depressed antioxidant and detoxification systems. concluded that, curcumin and its analogues are effective antioxidants which can protect human red blood cells from free radical-induced oxidative haemolysis and the H-atom abstraction from the phenolic group is responsible for the activity. The observations of, that the compounds bearing ortho-diphenoxyl functionality exhibit markedly higher anti-haemolysis activities than those bearing no such functionality gives us useful information for antioxidant drug design. demonstrated that curcumin has displayed a protective influence on the erythrocyte integrity in the high fat diet-induced hyperlipidemia.

The present study showed that the animals injected subcutaneous daily with 0.8 mg, nicotine/kg body weight concurrently with curcumin 20 g/kg diet and fenugreek seeds 7.5 g/kg diet daily for 30 consecutive days were caused a significant improvement in all hematological parameters when compared with nicotine group. These parameters were nearly similar to that in the control groups, that may be due to the additive antioxidant effect of fenugreek and curcumin together. reported that combined therapy with
both curcumin and quercetin was much better than each one alone. Because, previous studies reported that natural antioxidants strengthen the endogenous antioxidants defenses from reactive oxygen species and restore the optimal balance by neutralizing reactive species.[8, 9] Curcumin has anti-inflammatory and antioxidant properties with a potent ability to inhibit reactive oxygen species formation.[10] Curcumin represents a class of anti-inflammatory and anti-oxidant reported to be a potent inhibitor of reactive oxygen species formation.[11] Fenugreek had a different active constituents such as flavonoids, alkaloids, vitamins and amino acids.[12] The ameliorative effect of fenugreek and curcumin against nicotine induced hematotoxicity may be due to decrease nitric oxide production, uremic toxin, and increasing radical-scavenging enzyme activity through scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions.

**CONCLUSION**

It can be concluded that nicotine had a strong effect on the hematological parameters. The ingestion of fenugreek and curcumin may be useful in combating free radical-induced hematotoxicity induced by nicotine.

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