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AMELIORATIVE EFFECTS OF FENUGREEK SEEDS AND CURCUMINAGAINST HEMATOXICITY INDUCED BY NICOTINEIN MALE ALBINO RATS

Azab Elsayed Azab*[1], Mohamed Omar Albasha[2], Manal Abuelkasem Elnaif [3]
[1]Physiology Depatrment, Faculty of Medicine, Sabratha University, **Libya,**azabelsaied@yahoo.com
[2]ZoologyDepatrment, FacultyofScience, Alejalat, ZawiaUniversity, **Libya,** m.albasha@zu.edu.ly
[3]ZoologyDepatrment, FacultyofScience, ZawiaUniversity,**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 parameters 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.

Keywords :Nicotine, Hematotoxicity, Fenugreek and curcumin, Co-administration, Male albinorats

الملخص:

هدفت هذه الدراسة إلى تقييم التأثير المحسن لبذور الحلبة والكركم على السمية الدموية التي يسببها النيكوتين في ذكور الجرذان البيضاء. استخدم في هذا الدراسة 30 من ذكور الجرذان البيضاء (**F-344/NHsd fisher**) يتراوح وزنها من 180-200جم. وقسمت الحيوانات إلى خمس مجموعات (6 جرذان لكل مجموعة)؛ المجموعة الأولى(المجموعة الضابطة)، المجموعة الثانية (المجموعة المعاملة بالنيكوتين)، المجموعة الثالثة (مجموعة النيكوتين وبذور الحلبة معا)، المجموعة الرابعة (مجموعة النيكوتين والكركم معا، والمجموعة الخامسة (مجموعة النيكوتين والحلبة والكركم معا). في نهاية التجربة وبعد 24 ساعة من الجرعة الأخيرة تم

Author Correspondent:azabelsaied@yahoo.com

تخدير الحيوانات بالإيثير، وتم جمع عينات الدم من القلب. وجمعت في أنابيب نظيفة وجافة تحتوي علي مادة الإديتا المانعة للتجلط لاستخدامها في دراسة المتغيرات الدموية. أظهرت النتائج إن معالجة الحيوانات بالنيكوتين لمدة 4 أسابيع قد أدت إلي انخفاض معنوي(**P<0.01**) في عدد كريات الدم الحمراء والهيموجلوبين والهيماتوكريت ومتوسط الهيموجلوبين في كريات الدم الحمراء ومتوسط الهيموجلوبين في كل 100 ملي لتر من الدم وعدد الصفائح الدموية وزيادة في حجم كريات الدم الحمراء وعدد خلايا الدم البيضاء بالمقارنة مع المجموعة الضابطة. وقد أدي الحقن بالنيكوتين مع تناول الحلبة والكركم أو كليهما معا إلي حدوث تحسن في المتغيرات الدموية بالمقارنة مع مجموعة النيكوتين . نستنتج من هذه الدراسة ان للنيكوتين تأثير قوي علي المتغيرات الدموية وتناول الحلبة والكركم أو كليهما معا منع السمية الدموية التي سببها النيكوتين. وتقترح الدراسة الحالية ان الحلبة والكركم أفادو في مكافحة الجذور الحرة التي سببت الإجهاد التأكسدي والسمية الدموية التي سيبها النيكوتين.

الكلمات المفتاحية: النيكوتين، السمية الدموية، بذور الحلبة والكركم، ذكور الجرذان البيضاء.

1. INTRODUCTION

Cigarette smoking and the use of othertobaccoproductsbecame an important cause of increasedmortality and morbidity in developedcountries(Abdel-Aziz,2010), becauseitincreases the risk of heartdisease, diabetes, lung cancer, respiratorydisorders, and otherillnesses (Jessen*et al.,* 2003).

Nicotine is one ofhundreds of substances contained in cigarette smoke (Abdel-Aziz,2010). It is a highlytoxicorganic compound containingnitrogen and alkaloidwhichismostlyfound in tobacco (Jana *et al.,* 2010), and responsible for its addiction (Benowitz*et al.*,2009).

Nicotine induces a production of free radicals and consequentlyoxidative stress (Sanchez-Moreno *et al.,* 1999). Peoplewhosmoke and alsowho are exposed to cigarette smokeindirectly by breathing the air in the sameenvironment are exposed to nicotine inducedoxidative stress(Suleyman *et al.*,2002, and Ekinci*et al.,* 2010). Oxidative stress wouldresult in increased free radical injury in the tissue leading to extensive tissue damage withsubsequentderangement of cellphysiology (Abdel-Aziz,2010). As a consequence, theseradicalsinteractwithcell components such as lipids, proteins, DNA, RNA, carbohydrates and enzymes (Ekinci*et al.,* 2010, and Ekinci*et al., 2011*). So that smoking has an affect on the variousmetabolic and biologicalprocesses in the body (Abdel-Aziz,2010). Nicotine can easilypassthrough the cell membrane and react to tubulinproteinpresent in the cytoplasm of multiplyingcells and cause cell division disorder (Gorrod, 1993). It increases the risk of coronaryarterydisease (Swislocki*et al.*,1997), and promotestumorgrowth as well as atherosclerosisformation(Heeschen*et al.*,2001). Also, nicotine consumption can decreasefertility drive in males throughinducingoxidative stress and DNA damage (Jalili*et al.*,2014).

The body isengaged in a constant battle againstdamagingchemicalscalled free radicals or pro-oxidants to counter the harmfuleffects of free radicals, the body manufactures antioxidants to chemicallyneutralizethem. However, the naturalantioxidant system may not alwaysbeequal to the task. Sources of free radicals, such as cigarette smokemayoverwhelmthisdefensemechanism (EBSCO, 2007).

Natural antioxidantsstrengthen the endogenousantioxidantsdefenses and restore the optimal balance by neutralizingreactivespecies (Ho *et al.,* 1994). Curcumin as one of the

naturallyoccurringdietary substances has been usedsinceancient times for promotinghumanhealth (Joe *et al.*,2004). Curcuminis a major yellow pigments in rhizomes of *Curcuma longa*whichisusedwidely as a spice and coloring agent in severalfoods (Tirkey*et al.*,2005). It represents a class of anti-inflammatory and anti-oxidantreported to be a potentinhibitor of reactiveoxygenspecies (ROS) formation (Venkatesan*et al.*,2000).
Fenugreek (*Trigonellafoenumgraecum*) is an annualherbbelonging to Legumefamily;itiswidelygrown in India, Egypt, and Middle Eastern countries (Flammang*et al.*,2004). It usedboth in medicine and withfood as spice show antioxidanteffectthroughtheirused in diabetesmellitus due to the presence of different active constituentssuch as flavonoids, alkaloids, vitamins and aminoacids (Basch *et al.,* 2003).The yellowishseedscontain compounds withinterestingproprietieswhichexplaintheir use in variouswaysincludingmedicine, nutrition, beverages, fragrances, cosmetics, smoking and for otherindustrialpurposes (Djeridane*et al.*,2006). In fact, toasted and groundfenugreekseedis an essential ingredient of curry powders and isoften mixed withbreadstuffs (Blank *et al.,* 1997).

Plant seeds and herbs are used for treatments of diseases in the folk medicine. Their use wasincreased in manyfields due to theirsafetyness and itslowsideeffects as comparedwithchemicaldrugs (Alhawari,1986). Antioxidantpotential of curcumin and fenugreekseeds in the amelioration of nicotine inducedoxidative stress needthorough investigation becausethesenaturalantioxidants are components of manyedible substances and has the potential for safe future use by humans. The evidencereporting the protective effect of curcumin and fenugreekseedsagainst nicotine inducedhaemato-toxicity are hardlyfound.

2. OBJECTIVES

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

3.MATERIALS AND METHODS

3.1. Experimental Animals

Animals wereused in thisstudywere 30 male F-344/NHsd Fischer rats, weighingfrom 180 to 200g. Animals werepurchasedfrom Animal Welfare House of Libyan National MedicalResearch Centre, Zawia, Libya. Rats werekeptunder standard veterinaryhygienic conditions for cleanliness and health care and normal conditions through the wholeexperimentalperiods. Rats wereseparated in plastic cages, 6 rats per cage, and left one week of acclimation, beforecommencing the experiment. The rats werekept 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 ashwereused to feed the rats. Food and water weresupplied ad-libitum.

3.2. Methods and Technique

3.2.1.The Drug

Nicotine hydrogen tartrate salt (1-methyl-2-(3-pyridyl) pyrrolidine-bitartrate salt) waspurchasedfrom Sigma-Aldrich (St. Louis, MO, USA). Nicotine is a colorlessorganicLiquid. It wasdissolved in physiological saline (0.9% sodium chloride) and wasinjectedsubcutaneouslydailywith 0.8 mg, nicotine/kg body weight for 30 days.

3.2.2. Curcumin, and Fenugreekseeds

Curcuminwasgiven in diet as 20 g/kg dietdaily for 30 days.
Fenugreekseedswerefinelygrounded and added to the experimentaldiets as 7.5 g/kg dietdaily for 30 days.

3.3. 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 subcutaneously daily with 0.8 mg, nicotine/kg body weight for 30 days.

Group III (nicotine/fenugreek seeds co-administered): The animals were injected subcutaneously 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/curcuminco-administered): The animalswereinjectedsubcutaneouslydailywith 0.8 mg, nicotine/kg body weightconcurrentlywithcurcumin 20 g/kg dietdaily for 30 days.

Group V (nicotine/curcumin& fenugreek seeds co-administered): The animals were injected subcutaneously 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.

3.4.Blood Sampling:

At the end of the experimentation and 24 hoursafter the last dose, All animalswereanaesthetizedwithether and bloodsampleswerecollected by heartpuncture. The sampleswerecollected in a clean dry tube containing the anticoagulant substance EDTA (ethylene diamine tetra aceticacid) and used for the hematologicalstudies.

3.5.Determination of Haematological Parameters:

Red bloodcells count, haemoglobin concentration, hematocrit value, meancorpuscular volume, meancorpuscularhaemoglobin, meancorpuscularhaemoglobin concentration, white bloodcells count, differential count of leucocytes, and bloodplatelets count weredeterminedusing an automatedhaematologyanalyzerSysmex (KX. 21) machine.

3.6. StatisticalAnalysis: -

Resultswereexpressed as mean ± standard deviation, Data wereanalyzed by one way ANOVA. The differencebetweenmeans ± SD wastested at P<0.05 usingDuncan's multiple range test. In all statistical tests, the probabilitylevel of *P<0.05*wasconsideredsignificant.

4.RESULTS

4.1. Effect of administration of nicotine, and co-administration of nicotine withfenugreekseeds, nicotine withcurcuminand nicotine, fenugreekseeds, and curcumin on haematologicalparameters in male rats.

Haematologicalparameters of the different groups are shown in table .1 and figures (1-8). Male rats thatreceivedintraperitoneal injection of nicotine only (0.8 mg/kg body weight /day) for 30 consecutivedayshadsignificantly (*P<0.01*), decreasedRBCs 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 studyshowedthat the male rats injectedsubcutaneousdailywith 0.8 mg, nicotine/kg body weightconcurrentlywithfenugreekseeds 7.5 g/kg dietdaily for 30 consecutivedaysresulted 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& Figures 1-8). Conversely, co-administration of fenugreekseedswith nicotine significantly (*P<0.01*) improved all haematologicalparameterswhencomparedwith nicotine group Table.1& Figures 1-8).

Co-administration of 0.8 mg, nicotine/kg body weightsubcutaneouslywithcurcumin 20 g/kg dietdaily for 30 consecutivedayscaused 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 curcuminwith nicotine significantly (*P<0.01*) improved all haematologicalparameterswhencomparedwith nicotine group (Table.1& Figures 1-8).

The animalsinjectedsubcutaneousdailywith 0.8 mg, nicotine/kg body weightconcurrentlywithcurcumin 20 g/kg diet and fenugreekseeds 7.5 g/kg dietdaily for 30 consecutivedayswereshowed 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& Figures 1-8). Conversely, co-administration of fenugreek and curcuminwith nicotine significantly (*P<0.01*) improved all haematologicalparameterswhencomparedwith nicotine group Table.1& Figures 1-8).

Table.1: Effect of administration of nicotine, and co-administration of nicotine withfenugreekseeds, nicotine withcurcumin and nicotine withfenugreekseeds, and curcumin on haematologicalparameters in male rats.

Groups / Parameters	Control	Nicotine	Nicotine+ Fenugreek	Nicotine+ Curcumin	Nicotine+ Fenugreek+ Curcumin
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
RBCs ($x10^6$//µL)	9.7 ± 0.2	7.7 ± 0.4**	8.9 ± 0.1$^{**\#\#}$	8.6 ± 0.1$^{**\#\#}$	9.3 ± 0.1$^{\#\#}$
Hb (g/dl)	15.1 ± 0.2	11.9 ± 0.9**	14.3 ± 0.2$^{*\#\#}$	13.9 ± 0.2$^{**\#\#}$	14.8 ± 0.1$^{\#\#}$
HCT(%)	54.2 ± 1.2	43.5 ± 3.3**	50.4 ± 0.3$^{*\#\#}$	48.2 ± 0.7$^{**\#\#}$	51.5 ± 0.7$^{\#\#}$
MCV (μ^3)	52.5 ± 1.5	60.9 ± 1.5**	55.9 ± 0.5$^{**\#\#}$	57.4 ± 0.6$^{**\#\#}$	54.7 ± 0.3$^{**\#\#}$
MCH (pg)	17.0 ± 0.4	14.9 ± 0.2**	15.8 ± 0.1$^{**\#\#}$	15.3 ± 0.2$^{**\#\#}$	16.2 ± 0.1$^{**\#\#}$
MCHC (g/dl)	30.6 ± 0.8	26.3 ± 0.4**	27.9 ± 0.4$^{**\#\#}$	27.1 ± 0.3$^{**\#\#}$	29.1 ± 0.1$^{**\#\#}$
WBCs ($x10^3$//µL)	6.7 ± 0.7	14.4 ± 1.2**	10.7 ± 0.3$^{**\#\#}$	11.9 ± 0.6$^{**\#\#}$	10.1 ± 0.3$^{**\#\#}$
PLTs ($x10^3$//µL)	2021± 54	1282± 155**	1840± 65$^{**\#\#}$	1605± 83$^{**\#\#}$	1943 ± 37$^{**\#\#}$

*: Significant at (P<0.05) whencomparedwith control group, **: Significant at (*P<0.01*) whencomparedwith control group,
##:Significant at (*P<0.01*) whencomparedwith nicotine group.

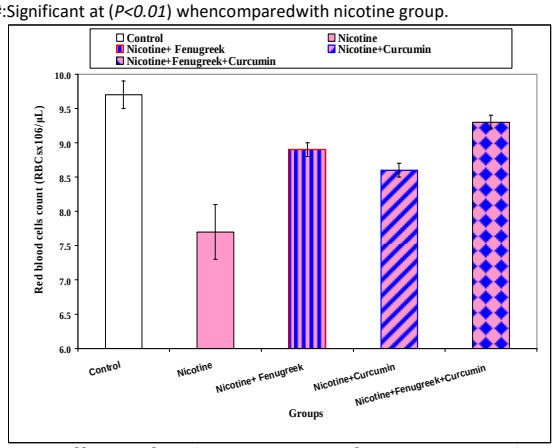

Fig.1: Effect of administration of nicotine, and co-administration of nicotine withfenugreekseeds, nicotine withcurcumin and nicotine withfenugreekseeds, and curcumin on RBCs count in male rats.

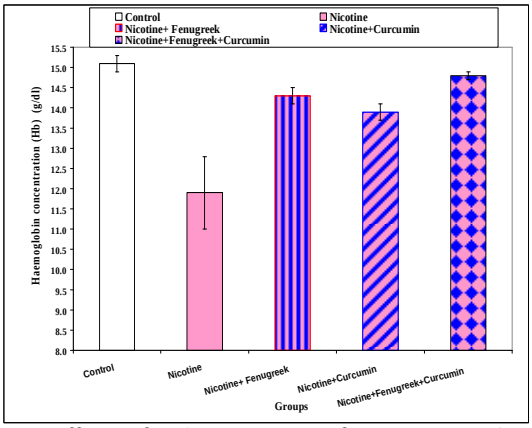

Fig.2: Effect of administration of nicotine, and co-administration of nicotine withfenugreekseeds, nicotine withcurcumin and nicotine withfenugreekseeds, and curcumin on haemoglobin concentration in male rats.

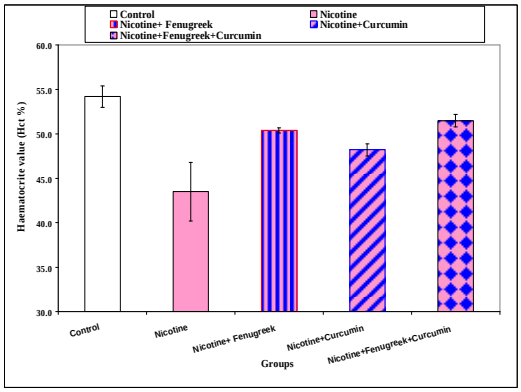

Fig.3: Effect of administration of nicotine, and co-administration of nicotine withfenugreekseeds, nicotine withcurcumin and nicotine withfenugreekseeds, and curcumin on hematocrit value (Hct) in male rats.

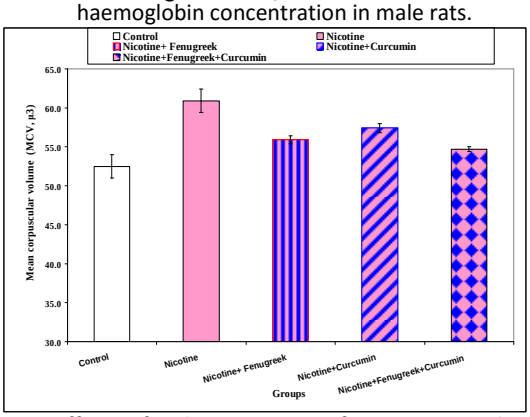

Fig.4: Effect of administration of nicotine, and co-administration of nicotine withfenugreekseeds, nicotine withcurcumin and nicotine withfenugreekseeds, and curcumin on meancorpuscular volume (MCV) in male rats.

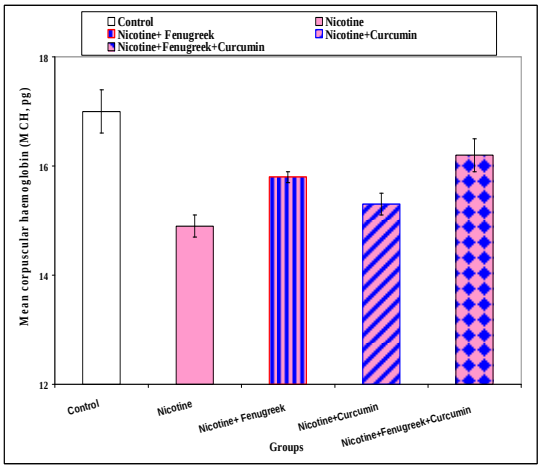

Fig5: Effect of administration of nicotine, and co-administration of nicotine withfenugreekseeds, nicotine withcurcumin and nicotine withfenugreekseeds, and curcumin on meancorpuscularhaemoglobin (MCH) in male rats.

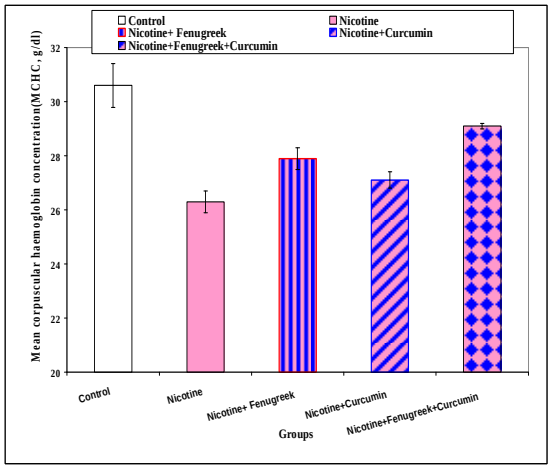

Fig.6: Effect of administration of nicotine, and co-administration of nicotine withfenugreekseeds, nicotine withcurcumin and nicotine withfenugreekseeds, and curcumin on meancorpuscularhaemoglobin concentration (MCHC) in male rats.

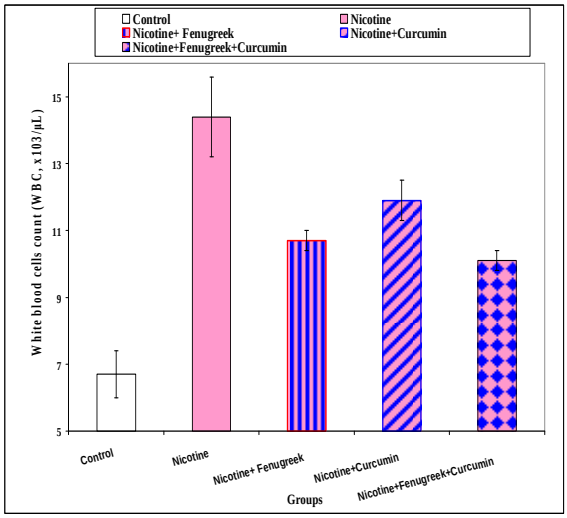

Fig.7: Effect of administration of nicotine, and co-administration of nicotine withfenugreekseeds, nicotine withcurcumin and nicotine withfenugreekseeds, and curcumin on WBCs count in male rats.

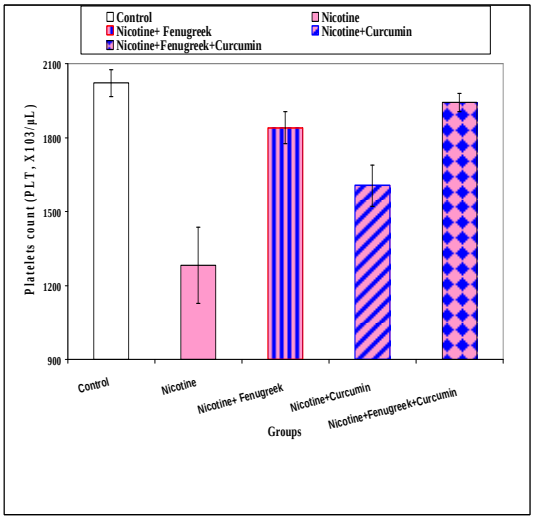

Fig.8: Effect of administration of nicotine, and co-administration of nicotine withfenugreekseeds, nicotine withcurcumin and nicotine withfenugreekseeds, and curcumin on platelets count in male rats.

5.DISCUSSION

In the currentstudy, male rats thatreceivedintraperitoneal injection of nicotine only (0.8 mg/kg body weight /day) for 30 consecutivedayshadsignificantly ($P<0.01$), decreasedRBCs count, hemoglobin concentration, hematocrit value, MCH, MCHC, and platelets count, and increased MCV and WBCs count as compared to the control group.Theseresults are similar to the study ofSharif*et al.*(2014) whoreportedthatmiceinjectedwith 1 mg/kg body weight of nicotine daily for 6 weekscaused a significant ($p\leq0.05$) increase in hematocrit, meancorpuscular volume, and white bloodcells, and a significantdecrease in RBCs count, meancorpuscularhemoglobin, hemoglobin, and meancorpuscularhemoglobin concentration comparedwith control group. Corre *et al.* (1971) recordedthat smokingcaused a significantincrease in WBCs count and a decrease in RBCs count. Also, nicotine causes many changes in bloodcells as itsimply diffuses into the cells (Rausch *et al.*, 1989 and Schwartz *et al.*, 2005). Also, Zafar *et al.* (2003) reportedthat cigarette smoking caused a significant ($p\leq0.05$) decreasedhemoglobinlevel. Okuno (1973) reportedthat smoking caused a significantincrease in MCV and asignificantdecrease in MCH and MCHC. The previousstudies (Sherwin and

Gastwirth, 1990, Siana*et al.*, 1992, and Sharif*et al.*,2014)showedthat nicotine administration caused a decrease in the proliferation of redbloodcells and as a result, the RBCs count decreases. Low erythrocytes count may lead to a number of physiologicaldisorders (Sharif*et al.*,2014). Nicotine greatlysuppresses the function of the immune system and due to thisreason the number of WBCsincreased in the body to strengthen the immune system. (Geng *et al.*,1996, Sharif*et* al.,2014).

Co-administration of fenugreekseedswith nicotine significantly (*P<0.01*) improved all haematologicalparameters, 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. Theseresults run parallel to thosereported by manypreviousstudies (Bravo, 1998, Effraim*et al.*, 1999, Algridi, and Azab, 2021). The study of Rosioru*et al.* (2010) reportedthattreatment of rats with 10% ethanol in drinking water for 30 dayscaused a significantincrease 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% fenugreekflour in the diet of ethanol-intoxicated rats for 30 daysshowed a tendency to restore the control values

Hamden *et al.* (2011) 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, (Kaviarasan, and Anuradha, 2007, Belaid-Nouira*et al.*, 2013).

Also, Abdel-Daim*et al.* (2014) 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.

Kandhare *et al.* (2015) reported that fenugreek seeds influenced the hemoglobin and lymphocytes count, improving hematopoietic function.

Al-Amri and Alrasheedi (2016) 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 increasing lymphocytes, suggesting that fenugreek seeds might improve immunity.

Abdel-Rahman *et al.* (2016) 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.

Pradeep and Srinivasan (2018) 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 by their antioxidant potential. Dietary fenugreek seeds and onion caused a decrease in glycated

haemoglobin (Pradeep, and Srinivasan, 2017), and a nephro-protective (Pradeep and Srinivasan, 2018) 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.

Elghazaly*et al.* (2019) 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, Algridi, and Azab (2021) recorded that treatment of male rabbits with aluminum chloride was decreased red blood cell count, hemoglobin concentration, haematocrite, 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 (Bravo, 1998, Rosioru*et al.*, 2010, 2016 Arivalagan*et al.*, 2013, Abdel-Daim*et al.*, 2014, Abdel-Rahman *et al.*, 2016), the antioxidant property of fenugreek inhibits lipid peroxidation of the erythrocytes (Thirunavukkarasu*et al.*, 2003), and the high iron content of fenugreek seed flour stimulated hemoglobin synthesis (Abdel-Rahman *et al*., 2016, Elghazaly*et al.*, 2019). 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 (Kaviarasan*et al.*, 2004). Fenugreek seeds may be improving immunity because they play a role in protecting the spleen and increasing the lymphocytes (Sindhu *et al.*, 2012, Kandhare *et al.* 2015, and Elghazaly*et al.*, 2019). Abdel-Rahman *et al.*, 2016 suggested that the administration of fenugreek powdered seeds were responsible for the 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 (Lawson et *al.*, 2005, Elghazaly*et al.*, 2019).

The currentstudyshowedthatco-administration of 0.8 mg, nicotine/kg body weightsubcutaneouslywithcurcumin 20 g/kg dietdaily for 30 consecutivedayscaused 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 curcuminwith nicotine significantly (*P<0.01*) improved all haematologicalparameterswhencomparedwith nicotine group. Theseresults run parallel to the results of Elsayed and Hegazi (2016) whoreportedthatmiceexposed to gasolinevapor2hours/day for 3 weeks in inhalation chambershowed a reduction in bonemarrowcellularity and slow rate of cells maturation. Apoptosisappeared in bonemarrowcells by histopathologicalexamination for biopsies. Also, reduction in a bloodcellcountswasoccurred, in RBCs, WBCs, platelets, and hemoglobin. Lymphocytes percentages in bloodweredepressed and neutrophils percentages wereelevated in gasoline inhalation group.All thesewereimproved andreturned to the normal levels by providingmicewithcurcumin in the diet.Curcuminprotectedleukocytesfromdepressioncaused by gasoline. This effect of curcumin on hematopoiesismaybe due to itsstronginhibitingeffect on myeloperoxidaseactivitywhichis the cornerstone enzyme in benzenehematotoxicity (Elsayed and Hegazi, 2016). The immunomodulatoryfunctions of curcuminhadappeared in the study of Antony *et al.* (1999), whenWBCs count, circulatoryantibodytiteragainstsheepRBCs, the plaque forming cells in the spleen,

significantlyincreasedwithcurcumin administration to Balb/c mice. Kato *et al.* (2003) estimatedthatcurcuminstronglyinhibitedmyeloperoxidaseactivity in vitro. Pal *et al.* (2005) recordedthatcurcumin administration to tumor-earingmicedecreasedtumorcellnumbersignificantly in a dose- dependentmanner. Furthermore, tumorinduceddepletion of immune cellnumber of the host, as wasevidencedfrom the decrease in bonemarrowprogenitor as well as thymic and splenicmononuclearcellnumberswasreinitiated by curcumin. Moreover, rather in tumor-bearingmiceitinhibitedhematopoietictoxicity, and activateddepressedantioxidant and detoxificationsystems. Deng *et al.* (2006) concludedthat, curcumin and its analogues are effective antioxidantswhich can protecthumanredbloodcellsfrom free radical-inducedoxidativehaemolysis and the H-atom abstraction from the phenolic group isresponsible for the activity. The observations of Deng et al. (2006) that the compounds bearing ortho-diphenoxylfunctionalityexhibitmarkedlyhigher anti-haemolysisactivitiesthanthosebearing no suchfunctionalitygive us useful information for antioxidantdrug design. Kempaiah and Srinivasan (2005) demonstratedthatcurcumin has displayed a protective influence on the erythrocyteintegrity in the high fat diet-inducedhyperlipidemia.

The presentstudyshowedthat the animalsinjectedsubcutaneousdailywith 0.8 mg, nicotine/kg body weightconcurrentlywithcurcumin 20 g/kg diet and fenugreekseeds 7.5 g/kg dietdaily for 30 consecutivedayswerecaused a significantimprovement in all hematologicalparameterswhencomparedwith nicotine group. Theseparameterswerenearlysimilar to that in the control groups, thatmaybe due to the additive antioxidanteffect of fenugreek and curcumintogether. Al Anany*et al.* (2015) reportedthatcombinedtherapywithbothcurcumin and quercetinwasmuchbetterthaneach one alone. Because, previousstudiesreportedthatnaturalantioxidantsstrengthen the endogenousantioxidantsdefensesfromreactiveoxygenspecies and restore the optimal balance by neutralizingreactivespecies (Albasha and Azab, 2014, Fetouh FA, and Azab, 2014). Curcumin has anti-inflammatory and antioxidantpropertieswith a potentability to inhibitreactiveoxygenspecies formation (Biswas*et al.,* 2005). Curcuminrepresents a class of anti-inflammatory and antioxidantreported to be a potentinhibitor of reactiveoxygenspecies formation (Venkatesan*et al.,* 2000). Fenugreekhad a different active constituentssuch as flavonoids, alkaloids, vitamins and aminoacids (Basch *et al.,* 2003). The ameliorativeeffect of fenugreek and curcuminagainst nicotine inducedhematotoxicitymaybe due to decreasenitric oxide production, uremictoxin, and increasing radical-scavenging enzyme activitythroughscavengingreactiveoxygen and nitrogenspecies and chelatingredex-active transition metal ions.

6. CONCLUSION

It can beconcludedthat nicotine had a strongeffect on the hematologicalparameters. The ingestion of fenugreek and/or curcuminprevent the hematoxicityinduced by nicotine. The currentstudysuggeststhatfenugreek and curcuminmaybeuseful in combating free radical-inducedhematotoxicityinduced by nicotine.

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