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Ameliorated Impact of Fenugreek Seed Extract on Some Blood Cellular and Biochemical Parameters in Female Albino Rats Exposed to Lead Acetate

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ABSTRACT

Lead acetate (LA) is considered as the common hazardous compund present in our habitat. It has a several diverse-organ toxicant effect in various healthy issues included diseases of the hepatic, renal, and other body systems. Fenugreek seeds (Trigonella foenum graecum L.) were used in curing medicine and as food supplement since ancient times. After that, their antioxidant and hepatoprotective properties have been studied. Therefore the presented was based on their biological properties of this plant and conducted to examine the protective impact of fenugreek seed extract upon LA toxicity on some blood cellular and biochemical parameters of female albino rats (Rattus norvegicus). Twenty eight female rats were randomly and equally divided into four groups each one contain seven rats as following; first group received basal diet and tap water ad libitum. Second group received basal diet and LA 80 mg/L with water ad libitum. Third group received basal diet contained 2.5% fenugreek seed extract and LA 80 mg/L with water ad libitum. Fourth group received basal diet contained 2.5%

fenugreek seed extract with water ad libitum. Rat's administration was continued daily for two weeks respectively. The administration of rats with LA produced significant reduction in liver weight, food intake, while it increased triglyceride (TG), alkaline phosphatase (ALP), aspartate aminotransferase (AST), uric acid (UA) and creatinie (CR). While supplemented diet with 2.5% Fenugreek seed extract improved body weight, organ weight, lipid peroxidation, TG, ALP, and AST. Our results plead for the profiteering of fenugreek seeds as a dietary supplement, because it showed protective effect of their content in polyphenolic flavonoids, antioxidant and membrane-protective effects, against exposure to the LA compound to protect injurious risk.

1. INTRODUCTION

Many medicinal plants were used as sources of many potent and powerful drugs around the world. Fenugreek is one of a self-pollinating crop belonging to the family of fabaceae that is leguminous herb cultivated in India and Middle East. It grows to an average height about two feet which can grow in dry and semi-dry area, also it has been used in food supplement for thousands of years beside their using in pharmacology as medicine [1]. Fenugreek is a medicinal plant that has been used as a treatment for number of disease, and used as the natural products administered by 80% of the people around the world as a medicines especially in the developing countries [1]. On the other hand, LA lead is one of the most environmental pollutant toxic [2] which derivate from petrol component in many countries, also many origins such as industrial pollution may include for their elimination [3]. lead exposure could occur during the manufacture of batteries, painting, printing, pottery glazing, and lead smelting processes. Furthermore, it occurs during the construction of tank padding, piping and other equipment such as electrical and communication wires [4].

2. LITERATURE REVIEW

It has been demonstrated that seed extract of fenugreek could be used in many treatments for a number of disorders such as atherosclerosis, rheumatism, drcreasing blood sugar elevation, blood lipids besides its antioxidant and antibacterial [5]. Besides that also the fenugreek seed extract could cause a decease total BW, the mechanism action fenugreek seed extract in decreasing of BW and adipose tissue is occurred by the flush-out the carbohydrate from the small intestine because its compound soluble fiber is about 40% could decline the digestion and absorption of food [6]. Lead acetate is a white crystalline compound analogous to other lead compound, highly poison and water soluble also is one of the non-essential elements of human body as well as the common path of potential human exposure to LA are inhalation, dermal contact and ingestion, which is also absorbed about 1.5 times more rapid than other lead combination by human [7]. Lead acetate toxicity of is closely in relationship to age, sex and many various forms of diseases have been associated such as cancer nephrotoxicity and central nervous system effects and

cardiovascular disease in human. Also the LA inhalation could adequately lower intelligence quotient damage stability and cause hyperactivity, and hearing loss. Accumulation of LA in human body produces degeneration effects in the hematopoetical, hematic, renal system and gastrointestinal tract [8]. The LA cause increase in BW by hepatic lipid accumulation and having effect on the DNA methylation which regulate the glucose and lipid metabolism genes and cause dysfunction of glucose metabolism pathway [9]. Previous studies showed that the exposure to lead cause damages to liver cells due to affecting cell membrane permeability [10]. Another study, reported that lead drive microcytic hypochromic anemia due to interference with copper and iron metabolism results in iron disruption [11]. It has been suggested that tri ethyl lead toxicity produce some hematological indices and has revealed a significant decrease in some haematological parameters included the mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and red blood cells (RBC) and an increase in monocyte number, and platelets [12]. The high dose of lead administration in female rats caused mild anemia, reduced MCH, MCV and mean corpuscular hemoglobin concentration (MCHC), erythrocytes enzyme activity declination and elevated of stippled RBCs [13].

3. METHODS AND MATERIALS

Animals housing

The female of inbred albino rats (*Rattus norvegicus*) [14] were produced from the animal house of Biology Department, Faculty of Science, Soran University, Soran, Iraq. The present study was conducted on twenty-eight animals. They were weighting 210 – 280 gm and 12 weeks of age at the time when the experiment started. They adapted in an environmentally planned room at steady temperature 22 ± 2 ℃, they were preserved at free access to tap water *ad libitu,* they fed at a standard pelleted chow feed according to Pico Lab. Rodent diet 20 . During the experiment the cages were wash out once a week.

Standard diet preparation

The component of diet were determined according Pico Lab. Rodent diet 20, with assistance of expert of in Erbil poultry project and Erbil animal diet factory Erbil, Iraq, as following; wheat 66.6%, soya 25.6%, Fenugreek 2.5%, salt 0.63%, lime stone 1.5%, methionine 0.156%, lysine 0.244%, choline chloride 0.062%, vit CX lay 0.058%, dicalcium phosphate 0.642%, AZ /1200= 0.080% and trace elements 0.050%.

Lead acetate dose preparation

The LA dose was prepared by dissolving 80 mg of LA (Spain) in 1 liter of tap water and put into bottles were given to animals replaced with water as *ad libitum*.

Fenugreek seed extract dose preparation

Our fenugreek seeds were gained from the Soran local markets. They cleaned then grounded by grinder in to fine powder. According to [15] with some alterations according to the method of [16] the fenugreek seed boiled aqueous extract powder was obtained by boiling 25 gm of seed powder in 250 ml of water about 30 minutes . Then the powder mixture was filtered out of eight layers of gauze and directly mixed with the standard rat's diet to obtain the supplemented diet containing fenugreek seeds aqueous extract of 2.5%.

Experimental design

Group I (control group): Animals were given basal diet and tap water *ad libitum.*

Group II: Animals were given standard diet and water contained LA (80mg/L) *ad libitum*.

Group III: Animals were given diet supplemented with fenugreek seed extract of 2.5% and water contained LA (80mg/L) *ad libitum.*

Group IV: Animals were given standard diet supplemented with 2.5% fenugreek seed extract and tap water *ad libitum*.

The experiment was continued daily for two weeks respectively.

Feed intake and body weight measurement

At the experiment beginning and the end of each of experimental week, the weight of animals was recorded in gm, and at the end of the two weeks the weights of standard-diet also were recorded in gm/rat.

Blood samples collection

After the twenty four hours of the end of treatment period the experimental animals were anesthetized with a combination of ketamine hydrochloride (90 mg/B.W.) and xylazine (10 mg/ B.W.) intraperitoneally [17]. Samples of blood were collected, by animal cardiac puncture and poured into chilled tubes for further measurement of some haematological and biochemical parameters.

Determination of spleen, liver and right kidney weight

After collecting blood sample collection animals are dissected. Some organs weight included liver, right kidney and spleen were recorded by precision electronic balances.

Blood cellular parameters measurement

The blood cellular parameters (haematological parameters) including Hb, RBC, WBC, PLTs, HCT and MCV were measured by coulter counter (Nihon Kohden, MEK-6410K, Japan) for each group [18].

Lipid profile measurement

Lipid profiles parameters including total amount of cholesterol (TC), low density lipoproteins (LDL), triglycerides (TG) and high density lipoproteins (HDL) were measured with cobas analyzer (c 111) .

Determination of serum malondialdehyde

Serum MDA concentration, was determined spectrophotometrically, 150 µL serum sample the followings were added, firstly, 1ml of 17.5% trichloroacetic acid (TCA) then, 1ml of 0.66% thiobarbituric acid (TBA) were mixed well by vortex mixer, after that they incubated in boiling water for 15 minutes, and then allowed to cool. Next that, one ml of 70% TCA was added and the mixture allowed to stand at room temperature for 20 minutes, finally, the mixture was centrifuged at 2000 rpm for 15 minutes, the supernatant was obtained for scanning spectrophotometrically at 532nm [19].

Measurement of liver function tests

Liver function tests including AST, alanine aminotransferase (ALT) and ALP were measured by cobas analyzer.

Measurement of kidney function tests

Kidney function tests including creatinine (CR) and uric acid (UA) were measured by cobas analyzer (c 111).

Statistical analysis of data

Our data were analyzed with one-way analysis of variance (ANOVA) by using statistical package for the social sciences (SPSS). The level of significant (probability level) fixed at $p<0.05$. The data expression was shown as mean ± standard error (mean ± S.E.) below each tables of the results and star symbol (*) means the significant differences.

4. RESULTS

Impact of lead acetate and fenugreek on body weight in female albino rats

The influence of fenugreek and lead acetate on BW in rats is shown in (Table 1). Rats administered with LA showed non-significant decline of BW in first week and second week as compared to the control group. While treated of rats with both lead acetate and fenugreek caused non-significant decrease of BW in first and second weeks as compared to LA group. Rats treated with fenugreek significantly ($p<0.05$) decreased of BW in first and second weeks as compared to the control group.

Table 1: Body weight

Parameter / Group	1st Week (gm)	2nd Week (gm)
Control	250.00± 9.258 [b]	264.29± 6.851 [b]
Lead acetate	225.71± 8.689 [ab]	235.71± 9.476 [ab]
Fenugreek and lead acetate	210.00± 11.126 [a]	221.43± 12.426 [a]
Fenugreek	208.57± 11.218 [a]	210.00± 10.465 [a]

Data are presented as mean ± S.E, the same letters mean non significant differences whereas the different letters mean the significant differences (*= p< 0.05)

Impact of lead acetate and fenugreek on liver, spleen and kidney weight in female albino rats

The influence of LA and fenugreek on some organs weight included liver, spleen and kidney in rats is shown in (Table 2). Rats administered with LA showed significant ($p<0.05$) decrease in liver weight, and non significant change of kidney and spleen weight as compared to the control group. Meanwhile treated rats with LA and fenugreek to rats showed non-significant increase of liver ,kidney, and spleen weight as compared to LA group. Also rats treated with fenugreek showed non-significant increase of kidney and spleen weight except liver significantly ($p<0.05$) increase as compared to the control group.

Table 2: Organs weight included liver, kidney and spleen

Parameter / Group	Liver (gm)	Kidney (gm)	Spleen (gm)
Control	7.171± 0.289[c]	0.795± 0.092[a]	1.185± 0.085 [a]
Lead acetate	5.307± 0.136[a]	0.748± 0.051 [a]	1.102± 0.117 [a]
Fenugreek and lead acetate	6.024± 0.346[ab]	0.752± 0.047 [a]	1.115± 0.090 [a]
Fenugreek	7.264± 0.286[b]	1.550± 0.826 [a]	1.194± 0.125 [a]

Data are presented as mean ± S.E, the same letters mean non significant differences whereas the different letters mean the significant differences (*= p< 0.05)

Impact of lead acetate and fenugreek on food intake in female albino rats

The influence of fenugreek and LA on food intake in rats is shown in (Table 3). Rats treated with LA showed significant ($p<0.05$) decrease of food intake in first week and second week as compared to the control group. Whereas rats administered with LA and fenugreek caused non-significant increase of food intake in first week and significant increase ($p<0.05$) in second weeks as compared to the LA group. Rats treated with fenugreek elevated food intake non-significantly in first week and decreased significantly ($p<0.05$) in second week as compared to the control group.

Table 3: Food intake

Parameter / Group	1st Week (gm)	2nd Week (gm)
Control	108.331± 1.994[a]	112.672± 0.881 [a]
Lead acetate	77.500± 1.945[b]	64.833± 1.579[b]
Fenugreek and lead acetate	84.666± 1.282[b]	74.666± 1.282[c]
Fenugreek	115.672± 4.630[a]	104.002± 4.618[d]

Data are presented as mean ± S.E, the same letters mean non significant differences whereas the different letters mean the significant differences (*= p< 0.05)

Impact of lead acetate and fenugreek on several hematological parameters in female albino rats

The influence of fenugreek and LA on some hematological parameters is shown in (Table 4). The treatment of rats with LA showed non-significant change in WBC,RBC,HB,PCV,PLT and MCV parameters as compared to the control group. Meanwhile administration of rats with both LA and fenugreek also caused non-significant change as a compared to LA group. Besides that, rats treated with fenugreek showed non-significant change in RBC,HB, PCV and MCV except the PLT, was decreased significantly ($p<0.05$) while WBC was significantly($p<0.05$) increase as compared to the control group.

Table 4: Haematological parameters

parameter / group	WBC (10^3/µL)	RBC (10^6/µL)	HB (gm/dL)	PCV (%)	PLT (10^3/µL)	MCV (fL)
Control	3.300± 0.762[a]	6.224± 0.365 [a]	12.160± 0.551 [a]	36.620± 2.862 [a]	565.6± 16.693 [a]	54.040± 4.875 [a]
Lead acetate	4.050± 0.705[ab]	6.200± 0.179 [a]	12.050± 0.489 [a]	35.516± 1.481 [a]	567.6± 14.298[a]	52.166± 1.215 [a]
Fenugreek and lead acetate	4.100± 0.953 [ab]	6.252± 0.293 [a]	12.320± 0.538 [a]	35.860± 1.411 [a]	573.2± 18.701[a]	53.480± 1.184 [a]
Fenugreek	6.216± 1.050 [b]	6.573± 0.258[a]	13.100± 0.500 [a]	36.233± 1.359 [a]	518.5± 6.902[b]	54.116± 0.611 [a]

Data are presented as mean ± S.E, the same letters mean non significant differences whereas the different letters mean the significant differences (*= p< 0.05)

Impact of lead acetate and fenugreek on serum lipid profile and malondialdehyde parameters in female albino rats

The influence of LA and fenugreek on serum lipid profile and MDA parameters is shown in (Table 5). Rats treated with lead acetate showed non-significant change of cholesterol,MDA, HDL and LDL parameters except significant ($p<0.05$) increase in TG level as compared to control group. While treatment of rats with both LA and fenugreek caused non-significant change of cholesterol, MDA , HDL and LDL except TG level significantly ($p<0.05$) decrease as compared to LA group. Furthermore, rats treated with fenugreek also showed non-

significant change of cholesterol , TG,HDL and LDL parameters as compared to control groups.

Table 5: Lipid peroxidation and lipid profile parameters

Parameter / group	Malondialdehyde (nmol/L)	Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Control	2.1583 ± 20185 [ab]	55.833± 1.249[a]	50.518± 3.602 [a]	44.666± 2.800 [a]	15.833± 3.015 [a]
Lead acetate	2.6172 ± 0.25877 [a]	58.285± 2.417 [a]	63.142± 2.052[b]	37.428± 3.902 [a]	13.000± 1.154 [a]
Fenugreek and lead acetate	2.1071 ± 03646 [ab]	56.571± 1.986 [a]	49.000± 4.680 [a]	37.857± 3.034 [a]	15.571± 0.528 [a]
Fenugreek	1.8741 ± 0.1393 [b]	55.285± 2.243 [a]	50.285± 2.705 [a]	37.714± 3.590 [a]	13.571± 2.348 [a]

Data are presented as mean ± S.E, the same letters mean non significant differences whereas the different letters mean the significant differences (*= p< 0.05)

Impact of lead acetate and fenugreek on function tests of liver in female albino rats

The influence of LA and fenugreek on liver function tests parameters is shown in (Table 6). Rats treated with lead acetate increased ALP and AST level significantly ($p<0.05$) and non-significantly decrease ALT as compared to the control group. Besides that, rats administered with both LA and fenugreek showed significant decrease ALP and AST parameters in except the ALT was non-significant increase as compared to LA group, furthermore, rats administered with fenugreek showed non-significant increase ALP while non-significant decrease ALT and AST parameters as compared to control group.

Table 6: Liver function tests parameters

Parameter / Group	ALP (U/L)	ALT (U/L)	AST (U/L)
Control	122.166± 5.653 [a]	39.3333± 2.848 [a]	71.000± 5.079 [a]
Lead acetate	146.857± 4.214 [b]	38.4286± 2.877 [a]	128.86± 8.076 [b]
Fenugreek and lead acetate	117.285± 2.382 [a]	42.7143± 2.781 [a]	71.428± 6.971 [a]
Fenugreek	124.142± 4.588 [a]	36.7143± 1.445 [a]	62.100± 1.516 [a]

Data are presented as mean ± S.E, the same letters mean non significant differences whereas the different letters mean the significant differences (*= p< 0.05)

Impact of lead acetate and fenugreek on function tests of kidney in female albino rats

The influence of LA and fenugreek on kidney function tests parameters is shown in (Table 7). Rats administered with LA showed significant increase ($p<0.05$) of UA and CR level as compared to the control group. Meanwhile, treatment of rats with LA and fenugreek together showed non-significant change in UA and CR parameters level as compared to LA group, furthermore, rats treatment with fenugreek also caused significant ($p<0.05$) increase in UA level and CR level as compared to the control group.

Table 7: Kidney function tests parameters

Parameter / Group	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	1.3552± 0.116 [a]	0.3698± 0.040 [a]
Lead acetate	2.2143± 0.565 [b]	0.6113± 0.038 [c]
Fenugreek and lead acetate	1.6501± 0.259 [ab]	0.5336± 0.035 [bc]
Fenugreek	2.0474± 0.130 [b]	0.5280± 0.019 [b]

Data are presented as mean ± S.E, the same letters mean non significant differences whereas the different letters mean the significant differences (*= $p< 0.05$)

5. DISCUSSION

The declined body weight (table 1) with LA administration is in agreement with the finding of studies of [20] who demonstrated that lead produced the reduction in growing rate in experimental animals when fed lead. It has been showed that the reduction in BW in lead encompassed toxicities in rats [21]. Whereas the BW gain in the animal treatment by fenugreek agreed by the finding of [22] who suggested that fenugreek extract appeared to motivate the food intake (table 3). It has been proposed that animals owned a continuous exposure with the heavy metals usually lose their BW [23]. The same decrement has been detected in the liver weight significantly, the decrease in liver (table 2) is not only that the outcome of a food decrement intake, but also, from immediate toxicity of the LA, perhaps by malabsorption of nutrients from toxic effects on the gastrointestinal tract or by inhibition of protein synthesis [24]. While non-significant increase in kidney weight may attributed to the initial increase the aggregation of lead in the form of nuclear inclusion bodies in the proximal kidney tubular cells, on the other hand he rapid proliferation of the kidney proximal tubules may be in result to injury by the heavy metal [25] and the non-significant change of spleen is supported by the finding of [26]. Besides that, the slight elevate of liver, kidney and spleen weight by fenugreek and LA treatment is supported with the finding of [27] who suggested that diet supplemented with fenugreek seeds reduce nephrotoxicity. In addition, the fenugreek influence was confirmed in the rats group treated with fenugreek alone. The significant decrement of food intake (table 3) by the LA consumption in the drinking water is confirmed by previous study who reported that male rats treated with LA (20 ppm) led to BW declination [28]. Our result is also supported by the attempt of [29] who reported that rats administered with LA led to decrease in food intake and BW. Meanwhile, the significant elevation in food intake in the second week of rats treated with fenugreek and LA is confirmed with the finding of [30] who suggested that male wistar rats administered with diet combined with fenugreek seed extract orally by gavage (10 and 100 mg/day per 300 g BW) showed significant elevation in feed intake and the cause the eating motivation. Furthermore to their physiological signals such as neurotransmitter modifications functions inwards the central nervous system, the feeding behavior is affect by perceptual reason and it depends predominately upon

palatability. Thus, the effects of fenugreek on food intake and motivation to eat might be related to the well-known aromatic properties of the seeds [31].The non-significant changes in haematological parameters (table 4) of rats with LA administration is confirmed with the result of [32] who suggested that rats administered with LA in 0.4% solution in water supply for 12 weeks showed non-significant changes in the haematological parameters. Also it confirmed by [29] who suggested that rats treated with 0.1% of LA caused non-significant changes in Hb, MCV and HCT. Furthermore the non-significant changes of same parameters in rats treated with LA and fenugreek is confirmed by our previous study stated that rats treated with diet supplemented with fenugreek showed non-significant changes in blood cellular parameters [16]. The non-significant elevation of serum MDA with LA administration in our study was agreed by the finding of [33], who demonstrated that male rat's treatment with LA (500 mg/L) induced the oxidative damage in kidney and liver of rats. Current confirmation has reported the crucial role of lipid peroxidation pattern in the regulation of psychiatric diseases related processes, including accelerated aging, inflammatory response and neurodegeneration consequences [34]. On the other hand antioxidant therapy is the most reducing agents of the extent of free radical-mediated tissue damaging represent a rational approach in preventing the onset and/or progression of free radical related to the debilitations. It has been reported that free radicals and oxidative stress perform an important role in the pathogenesis of induced toxicity and its later consequences. The fenugreek seeds extracts acted in MDA reducing against LA toxicity and agreed with the result of [35] who suggested that rats administered fenugreek seeds showed lipid peroxidation level declination. Besides that, whole fenugreek seeds contain 48% total fiber, which included 20% gum and 28% neutral detergent fiber and about 4% of saponins [36]. The level of HDL is known as good healthy cholesterol in the blood stream in order to transports cholesterol to the liver to be expelled from the body. It helps to eliminate excess cholesterol from the body. The deprivation of HDL in our result is associated with elevation of both TG and cholesterol in the group of rats administered by LA, meanwhile, we observed the significant differences in the lipid fraction, and this is supported by previous study on atherogenic issue impacted by low doses of lead [37]. in addition, the increased levels of lipids is observed along with increased levels of liver function markers may attribute to the liver dysfunction [38] also our results are in accordance with the results of [39].The present study showed the significant elevation in ALP and AST with lead acetate administration These observations are in agreement with the study which reported that rats treated with gradual doses of LA lead to an increase of AST level [8, 40] this alteration may be due to the membrane lipids peroxidation of smooth endoplasmic reticulum and it confirmed with slight elevation of serum lipid peroxidation (MDA). Fenugreek seed extract treatment was minimized the effects of lead acetate in elevated liver enzymes. The beneficial effects of fenugreek seeds are well demonstrated by their ability to improve antioxidant status thereby lowering lipid peroxidation slightly. The antioxidant role of some dietary compounds such as polyphenols and flavonoids of plant origin have been experimentally proved. Physiological studies reported beneficial impacts related to consumption of such compounds [41]. In contrast, fenugreek seed extract declined the lead acetate toxicity, it lowered the elevated level of lipid fractions, it confirmed by the finding of [16, 35, 42]. The influence of LA on some kidney function test caused an increase in the uric acid and CR level. The renal parameters are highly responsive sensitive to the action of poison because of its intensive metabolic activity. Our results are confirmed by result of [43] who suggested that rats administered with lead acetate by orally through the drinking water to adult rats (aged three months) at the rate of 0.3% for 15 executive days lead to creatinemia, on the other hand the significant increase of UA of rats treated with LA is agreed with the attempt of [44] who demonstrated that mice were administered with LA for 28 days at three levels caused significant decrement of UA and CR. they are considered the most final breakdown product of purine metabolism and as a sensitive to the stress and tissue injures this confirmation was noticed by MDA level. Meanwhile the none significant increase of both mentioned renal parameters (UA and CR) of rats treated with both LA and fenugreek is confirmed by the finding of [45] who proposed that male rats treatment with fenugreek against

other toxic substances caused increase in the renal function parameters and the current protective impact is commonly referred to their antioxidant properties in fenugreek seed extract components.

6. CONCLUSION

Our study result and their interpretations concluded that, the LA declined of BW, food intake, and liver weight. Also it elevated TG level and produced many serological toxicity in the rats include liver and kidney function tests. On other hand rats administration with fenugreek seed extract showed some recovery of against LA such as weight gain of whole body and absolute organ weight. Fenugreek seed extract produced beneficial properties against lead acetate toxicity.

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