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Dietary polyethylene inclusion affecting rabbit's performance

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Abstract

A feeding experiment was conducted with rabbits as experimental animals for 2 months at 4 treatment groups under the same environmental conditions. The aim was to study the toxic effects of dietary graded levels of polyethylene (PE, 0, 1, 2, and 4 g/ton diet). The main effects were that some animals fed the PE-contaminated diets suffered from mange, diarrhea, weakness, and/or loss of weight. Post-mortem examination of the contaminated-diets fed rabbit revealed presence of bad smell liquids in its abdomen, residues of collective PE in the small intestine, collective feces in one part of the intestine and the parts were filled with aqueous accumulation, the color of one hepatic lobule was pale, and the lungs' color was bloody red. Yet, feed intake and water consumption increased by feeding the contaminated diets. Moreover, animals offered the contaminated diets reflected heavier body weight. However, the feed conversion (utilization) was negatively affected by the PE- contaminated diet; since, dietary PE-inclusion increased apparently feed consumption than the real body weight gain. Slaughter test of the PE-treated animals reflected many remarks including loss of rabbit's weight; increased skin weight, skin was thick and dough, changes in the color and quality of the carcass flesh, easy broken bone. When these animals were eviscerated, it were observed white-colored lungs, with necrosis and bloody infiltration, small-sized liver, with blackly-red color, enlarged gall bladder, narrow diameter of the small intestine, presence of collective mass of PE on the internal wall of the small intestine. The presence of PE in the experimental diets was responsible for significant lowering the specific gravity of the treated animals' bone, as well as their flesh moisture and fat; yet, their crude protein increased besides their edible parts and boneless meat percentages increased too, but not significantly. Dietary contamination with PE reflected its residues in the rabbits' flesh and increased the flesh content of ash ($P>0.05$). The contaminated diets were responsible for significantly ($P\leq0.05$) evaluating lymphocytes %, MCV, MCH, and PLT and lowering both monocytes and granulocytes % as well as RBCs count. Significant ($P\leq0.05$) increases in albumin, creatinine and uric acid concentrations and decreases of ALT activity were recorded with feeding rabbits the PE-contaminated diets; but, Cho. and Trig. concentrations were decreased ($P\leq0.05$). There was a significant increase of scores of hepatic congestion and necrosis in liver from rabbits group received 0.4 PE when compared with control group. Statistical analysis shows significant increase of scores of renal congestion, fibrosis and acute tubular necrosis in group received 0.4 PE when compared with control group. Hence PE negatively affected rabbits' performance, health and quality, and may affect the consumers' health too.

Keywords: Polyethylene, rabbits, post-mortem, performance, slaughter, chemical composition, residues, blood, histology.

1. Introduction

Polyethylene (PE) is used in different industries [1]. One of the main problems of polyethylene is that it is not readily biodegradable, and thus accumulates. A large rate of plastic wrapping goes to waste [2]. Moreover, the bacterium *Brevibaccillus borstelensis* and *Acinetobacter* sp. were found to use low-density polyethylene as a sole carbon source; when incubated together at 50 ºC. Since, biodegredation increased with time exposed to ultraviolet radiation [3]. Plastics processing and recycling pollute the environment with the organochlorides as dioxins [4].

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The holy Quran told us that such pollutants are due to human activities, as mentioned before 1440 years in the following meaning: In the name of Allah, the Compassionate, the Merciful, "Corruption has appeared on land and sea, because of what people's hands have earned, to make them taste some of what they have done, so that they may return", Great truth of God (Quran - The Romans – 41).

Since household is the main supplier of the plastic wastes, particularly of PE [5]. The plastic materials represent today an important category of waste. Most of them are re-used. In the concrete production these fibers are currently used for obtaining high strength concretes, shotcrete, self-compacting concrete, etc. [6]. The increase of population and the development of industrial processes, all these resulted in a higher consumption of more wastes and a higher pollution [7]. Recycling has therefore become a reasonable solution to the landfill problem [8]. It harms humans and animals alike. When it is burned, unhealthy smoke is a result. Rubbish causes flooding, air pollution and many other public health problems [9]. Plastic waste is a major cause of marine pollution [10].

Therefore, the present research aimed to evaluate the possible toxic effects on rabbits, concerning their growth performance, post-mortem examination, edible parts, chemical composition, residues of polyethylene, blood profile, and histological alteration in their liver and kidney.

2. Materials and Methods

Twenty four red "Balady" male rabbits of an average initial body weight 850 g were purchased from Elserw animal breeding station belonging to the Animal Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. The animals were housed in wire batteries, each of 4 flat cages (the diameters of each cage were 50x50x30cm) provided with feeders and drinkers. Each treatment was divided into 2 replicates; each replicate represented 3 rabbits in one cage. A thermometer and a hygrometer were found in the rearing room for daily measuring both air temperature and relative humidity surrounding the rabbits. The rabbits were adapted for 6 days on a basal diet, thereafter were offered the experimental diets for 2 months (from the 11th May till the 9th July 2019). The experimental diets were the basal diet (consisted of alfalfa 32%, barley 23%, wheat bran 18%, yellow 12%, soya bean 12%, calcium di-phosphate 1.6%, lime stone 0.8%, common salt 0.3%, and premix 0.3%) with replacing 0, 0.1, 0.2, and 0.4% of the diet with PE instead of the same % from the dietary calcium di-phosphate.

2.1. Slaughter test and blood and muscles' sampling

At the end of the experimental period, 3 rabbits/treatment were randomly chosen, fasted for 12 hours, slaughtered, eviscerated after removal of both head and skin, then the carcasses were divided into different parts to be individually weighed, and both of boneless meat and dressing percentages were calculated. Collective flesh samples (representative for the 4 quarters and the back muscles) were individually (from each of the 3 animals/treatment) taken. Meanwhile the slaughtering, blood samples from each rabbit were collected (from the jugular vein) for analysis. Samples of liver and kidney were taken and preserved in neutral formalin 10% for histological examination.

2.2. Analytical methods

Meat samples were undertaken for chemical analysis using FOSS NIRS TM DA 1650, Denmark, besides the quantitative determination PE-residues [11]. Adequate amount of whole blood was withdrawn in small plastic vials containing EDTA (ethylene-diamine tetra acetic acid) as anticoagulant and used to obtain the blood plasma by centrifuge at 3500 rpm for 15 min. Blood plasma samples were used for determination of creatinine [12], triglycerides [13], total proteins [14] and albumin [15] concentrations as well as the activity of aspartate amino transferase (AST) and alanine amino transferase (ALT) using commercial test kits (Humalyzer 3000 manufactured by Human, Germany). Globulin level was calculated by subtracting albumin from total protein. The other samples of blood were used to determine the blood hematology as concentration of hemoglobin (Hb), total count of erythrocytes (RBCs), and total leukocytes (WBC_S) [16] and hematocrit (Hct) using Auto Counter (920 EO+ manufactured by Swelab, Switzerland) [17]. The other hematological parameters were mathematically calculated. For histological examination, liver and kidney samples were imbedded in paraffin wax, sectioned at 5-6 μm via microtome, fixed in neutral

formalin 10% for 72 hours, immersed 3 times in ethanol alcohol 70%, dried in graded ethanol, then dyed with haematoxylin and eosin for examination under light microscope.

2.3. Statistical analysis

All obtained data were analyzed using one-way analysis of variance according to statistical analysis system software [18] for windows. Kruskalis-Wallis test was used for statistical analysis of the histopathological scores in the examined H&E stained liver and kidney sections. Multiple range tests [19] were used to compare between the parameters of the different nutritional groups. The differences were significant at 0.05 levels.

3. Results

3.1. Surrounding conditions:

Daily measuring both air temperature and relative humidity surrounding the rabbits revealed their ranges as 22-29ºC and 58-76%, respectively throughout the entire experimental period. Both measurements' values were increased through summer months (June and July) comparing with their values during May (the experiment begin).

3.2. Daily remarks

Some animals of the 2nd and 4th groups (fed with 0.1 and 0.4% PE, respectively) suffered from mange, diarrhea, weakness, and/or loss of weight. One rabbit from the 3rd (fed with 0.2% PE) died, its post-mortem examination revealed presence of bad smell liquids in its abdomen, residues of collective PE in the small intestine, collective feces in one part of the intestine and the parts were filled with aqueous accumulation, the color of one hepatic lobule was pale, and the lungs' color was bloody red.

3.3. Experimental diets

Table 1 presents the formulation of the experimental diets that differ only in the level of PE inclusion which replaced the same level (0, 0.1, 0.2, and 0.4%) from the dietary calcium di-phosphate.

Table 1. The formulation (%) of the experimental diets

Ingredients	% of PE			
	Control (0.0)	**0.1**	**0.2**	**0.4**
Yellow corn	12	12	12	12
Wheat bran	18	18	18	18
Barley	23	23	23	23
Soya bean	12	12	12	12
Alfalfa	32	32	32	32
Calcium di-phosphate	1.6	1.5	1.4	1.2
Lime stone	0.8	0.8	0.8	0.8
Common salt	0.3	0.3	0.3	0.3
Premix	0.3	0.3	0.3	0.3

3.4. Feed consumption

Means ± standard errors of feed consumption for rabbits of the four experimental groups at 10-day intervals are illustrated in Fig. 1. The figure shows significant ($P \leq 0.05$) differences among treatments, in favor of the PE-containing diets (particularly group 2) against the control.

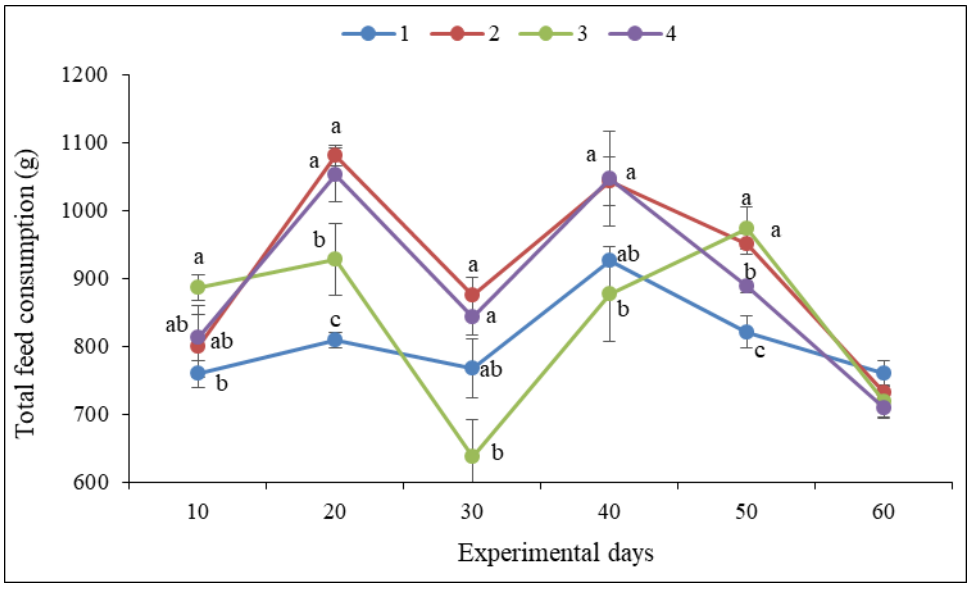


Fig. 1. Total feed consumption (g/rabbit/10 days as means ± standard errors) throughout the 60-days experimental period for the 4 treatment groups (a-b: means in the same column superscripted with different letters, significantly (P≤0.05) differ).

3.5. Drinking water

Average daily water consumption (ml/rabbit) was increased significantly (P≤0.05) by PE-inclusion in the experimental diets (Fig. 2) comparing with control. At the end of the experiment, group 2 represented the significantly (P≤0.05) highest total drinking water consumption throughout the entire experimental period (Fig. 3).

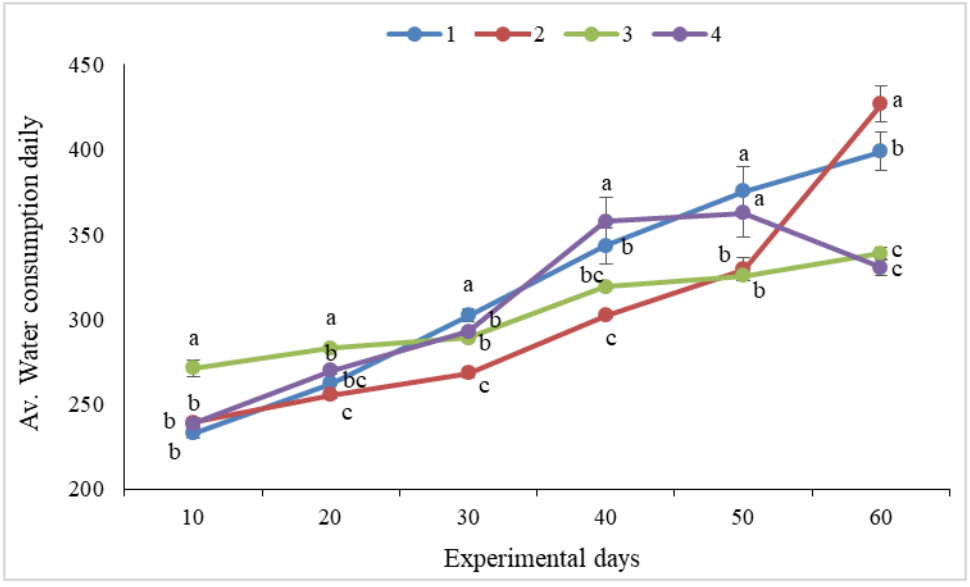


Fig. 2. Average drinking water consumption (ml/rabbit, as means ± standard errors) throughout the 60-days experimental period for the 4 treatment groups (a-c: means in the same column superscripted with different letters, significantly (P≤0.05) differ).

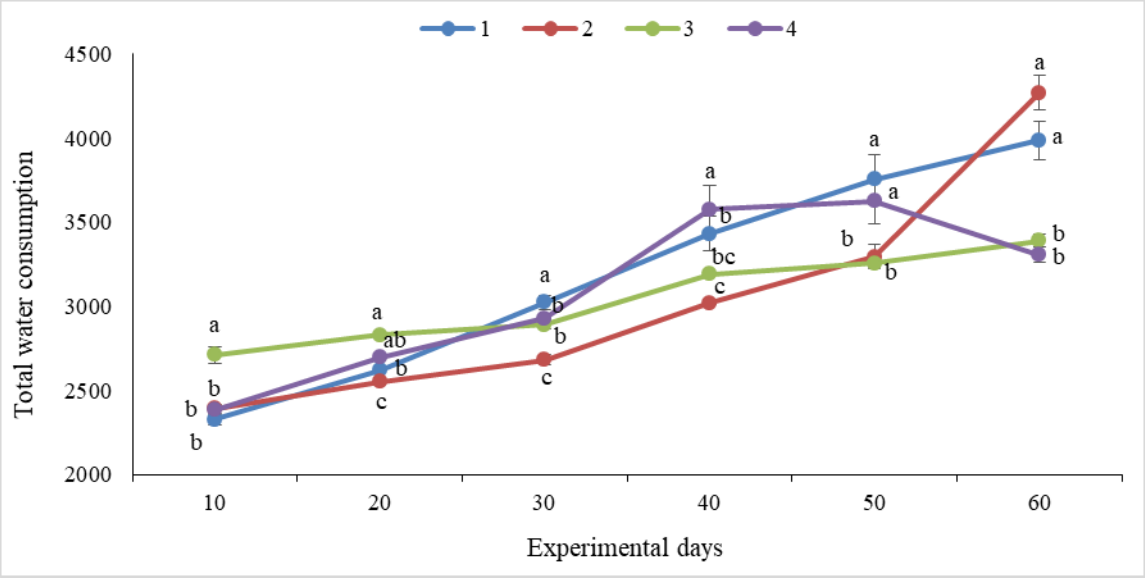


Fig. 3. Total drinking water consumption (ml/rabbit/10 days as means ± standard errors) throughout the 60-days experimental period for the 4 treatment groups (a-c: means in the same column superscripted with different letters, significantly (P≤0.05) differ).

3.6. Growth performance

Figure 4 illustrates body weight changes of the rabbits during the experimental period. Although the prescribed clinical symptoms registered in the treatment groups fed the PE-contaminated diets, and rabbits fed the three contaminated diets lost from their body weight at the last interval; yet, animals offered the contaminated diets reflected heavier body weight, particularly those in group 2 (for their higher feed consumption, Fig. 1).

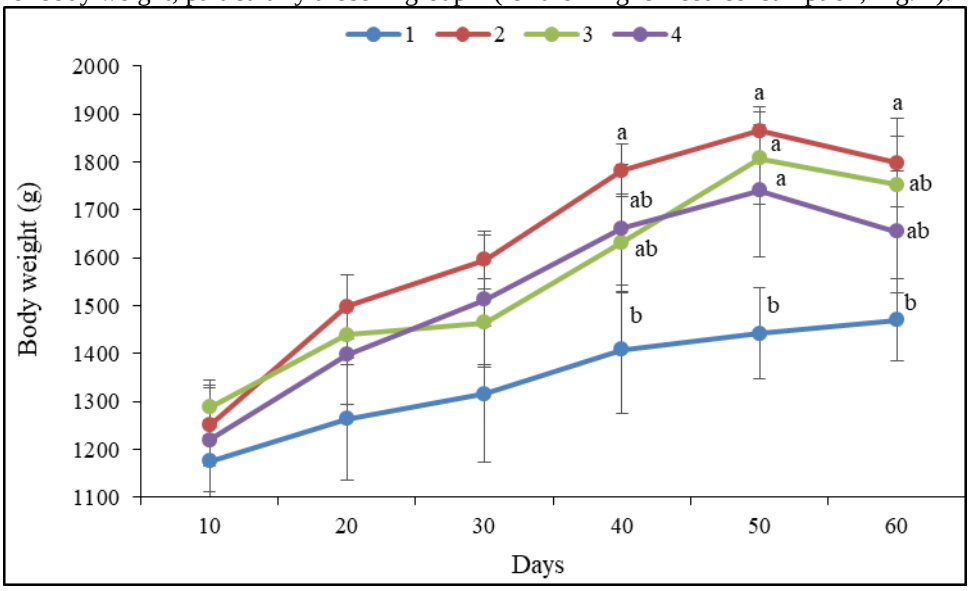


Fig. 4. Body weight (g/rabbit, as means ± standard errors) throughout the 60-days experimental period for the 4 treatment groups (a-b: means in the same column superscripted with different letters, significantly (P≤0.05) differ).

Although the loss of body weight in some experimental rabbits in all the experimental groups and intervals; yet, group-2 rabbits realized the significantly (P≤0.05) highest total body weight gain and group-3 animals reflected the best (but not significant, P>0.05) total average body weight gain (Fig. 5).

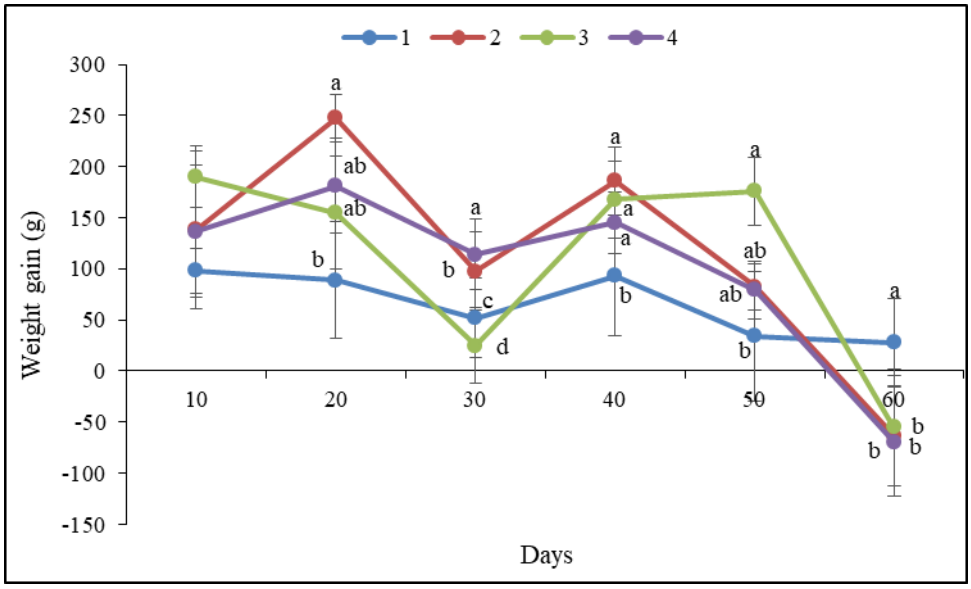


Fig. 5. Body weight gain (g/rabbit, as means ± standard errors) throughout the 60-days experimental period for the 4 treatment groups (a-d: means in the same column superscripted with different letters, significantly (P≤0.05) differ).

3.7. Feed utilization

Table 2 shows the feed conversion ratio for the experimental rabbits' groups as means ± standard errors for the entire period (60 days) as affected by the experimental treatments. However, the feed conversion (utilization) was negatively affected by the PE- contaminated diet; since, dietary PE-inclusion increased apparently feed consumption than the real body weight gain.

Table 2. Effect of the dietary treatments on the experimental rabbits' feed conversion ratio (mean ± standard error) throughout the entire period

Group No.			
1	**2**	**3**	**4**
5.27	8.34	8.41	10.21
±4.14	±1.16	±0.99	±1.53

3.8. Slaughter test

At the end of the experiment and during slaughtering the PE-treated animals, many remarks were recorded including loss of rabbit weight; increased skin weight relatively to the rabbit weight, skin was thick and dough, changes in the color and quality of the carcass flesh, since its meat took the pale yellow color besides its juicy consistency (not acceptable), easy broken bone (fragility, may be for no utilization of dietary nutrients and minerals). When these animals were eviscerated, the following findings were observed: white-colored lungs, with necrosis and bloody infiltration, small-sized liver, with blackly-red color, enlarged gall bladder, narrow diameter of the small intestine, and there for its size was also small.

Presence of collective mass of PE on the internal wall of the small intestine, making indentations (swellings) along the small intestine (like a long nicked balloon that may reduce or block the utilization of the absorbed nutrients for the harmful effect of the PE accumulation on the villi growth that negatively affected its functional role in the nutrients absorption, hence negatively affected the growth performance), so these rabbits reflected: muscles dystrophy and bone proses, for low absorption of the dietary protein, calcium, and vitamin D; pale colored muscles, for low iron

absorption, and hence low muscular myoglobin; weak growth (low weights) of the internal organs (liver, heart, kidneys, small intestine); general illness and low vitality, and consequently the animals were susceptible for external parasites (mange); and skin tended to be heavier, thick and dough.

Bad carcass properties and meat quality, those confirmed that dietary contamination with PE is very danger, negatively affected rabbits' growth performance and their dietary nutrients utilization, which harmed the animals' health, fertility, reproduction, psychology, nutritional physiology, and internal organs' functions, besides the bad effect on the economic production of rabbits. Severity of these negative effects was positively corresponding with the PE level in the diets; therefore, the 4th diet was the worst. So, if PE is used to cover the dietary pellets (to conserve the pellets quality and form), it will be a commercial and industrial deceit in favor of the producer but it harms the animals' health and their economic production.

3.9. Physical and chemical analysis of the carcass

As shown from Table 3, the presence of PE in the experimental diets was responsible for significant (P≤0.05) alterations, included lowering the specific gravity of the treated animals' bone, as well as their flesh moisture and fat; yet, their crude protein increased gradually and significantly (P≤0.05) besides their edible parts and boneless meat percentages increased too, but not significantly (P>0.05). Dietary contamination with PE reflected its residues in the rabbits' flesh (0.397-0.433%) and increased the flesh content of ash (P>0.05).

Table 3. Effect of the dietary treatments on some physical and chemical parameters for the experimental rabbits (mean ± standard error), at end of the experimental period

Items	**Group's No.**			
	1	**2**	**3**	**4**
Bone density, g/cm^3	1.03^{a} ± 0.027	0.71^{b} ± 0.047	0.69^{b} ± 0.030	0.53^{c} ± 0.122
Polyethylene residues in flesh, %	------	0.407 ± 0.034	0.397 ± 0.049	0.433 ± 0.110
Edible parts (fore and hind quarters, liver, kidneys, heart, dorsal muscle), %	53.0 ± 1.07	58.0 ±2.48	57.8 ±1.93	57.4 ± 0.99
Boneless meat, %	33.6 ± 1.30	38.6 ±3.24	41.1 ±1.73	37.4 ± 1.32
Flesh moisture, %	65.21^{a} ± 2.66	57.84^{ab} ±2.71	50.48^{b} ±1.95	57.22^{ab} ±4.22
Flesh crude protein, % dry matter basis	71.59^{c} ±10.48	77.52^{ab} ±5.15	81.56^{a} ±3.69	82.19^{a} ±1.75
Flesh crude fat, % dry matter basis	20.74^{a} ±9.24	19.17^{a} ±3.68	14.52^{b} ±2.97	14.11^{b} ±1.22
Flesh ash, % dry matter basis	7.90 ±0.99	9.75 ±0.35	8.67 ±0.39	9.72 ±0.47

a-c: means in the same row superscripted with different letters, significantly (P≤0.05) differ.

3.10. Hematological parameters

Although the white blood cells count did not significantly (P>0.05) affected, PE-contaminated diets were responsible for significantly (P≤0.05) evaluating lymphocytes % and lowering both monocytes and granulocytes % (Table 4). The RBCs count was significantly (P≤0.05) decreased but MCV, MCH, and PLT were increased by feeding with the contaminated diets (Table 5).

Table 4. Effect of the dietary treatments on the experimental rabbits' white blood cells (mean ± standard error), at the end of the experimental period

Group	WBCs*	Lymphocyte (%)	Monocyte (%)	Granulocyte (%)
1	10.08 ±0.98	18.72^{b} ±1.76	19.10^{a} ±0.76	62.18^{a} ±2.46
2	13.37 ±1.93	55.63^{a} ±13.25	11.42^{c} ±0.45	32.95^{d} ±13.14
3	10.03 ±1.29	26.83^{ab} ±7.25	14.70^{b} ±0.76	58.47^{b} ±7.92
4	9.63 ±0.26	38.65^{ab} ±10.88	15.65^{b} ±0.69	45.70^{c} ±10.20

*WBCs: white blood cells count, X 10^3/µl. a-d: means in the same column superscripted with different letters, significantly (P≤0.05) differ.

Table 5. Effect of the dietary treatments on the experimental rabbits' hematology (mean ± standard error), at the end of the entire experimental period

Group	RBCs	Hb	HCT	Blood indices			PLT
				MCV	MCH	MCHC	
1	6.12^{a} ±0.16	11.87^{ab} ±0.44	34.18 ±1.03	55.87^{b} ±0.61	19.37^{b} ±0.32	34.68 ±0.25	334.5^{b} ±32.31
2	5.75^{b} ±0.17	11.73^{ab} ±0.42	34.63 ±1.14	60.13^{a} ±0.31	20.35^{b} ±0.16	34.33 ±0.59	416.2^{a} ±14.70
3	5.67^{b} ±0.03	12.70^{a} ±0.44	36.98 ±1.47	63.42^{a} ±2.29	21.73^{a} ±0.70	34.33 ±0.29	370.0^{ab} ±24.56
4	5.57^{b} ±0.04	11.32^{b} ±0.15	33.92 ±0.56	60.28^{a} ±0.75	20.32^{b} ±0.20	33.70 ±0.45	401.0^{ab} ±11.15

RBCs: red blood cells, X 10^6/µl; Hb: hemoglobin, g/dl; HCT: hematocrit, %; MCV: mean corpuscular volume, fl; MCH: mean corpuscular hemoglobin, pg; MCHC: mean corpuscular hemoglobin concentration, %; PLT: platelets, X 10^3/µl. a-b: means in the same column superscripted with different letters, significantly (P≤0.05) differ.

3.11. Plasma biochemical parameters

Significant (P≤0.05) increases in albumin, creatinine and uric acid concentrations and decreases of ALT activity were recorded with feeding rabbits the PE-contaminated diets (Table 6); but, Cho. and Trig. concentration were decreased (P≤0.05) as shown from Table 7.

Table 6. Effect of the dietary treatments on the experimental rabbits' plasma biochemical (liver and kidney functions) parameters (mean ± standard error), at the end of the entire experimental period

Groups	TP	AL	GL	AST	ALT	Crea.	U. acid
1	9.13 ±0.12	3.83^{c} ±0.09	5.30 ±0.17	56.03 ±5.28	64.28^{ab} ±2.93	0.85^{b} ±0.02	0.90^{b} ±0.04
2	8.33 ±0.75	4.13^{b} ±0.06	4.20 ±0.72	56.57 ±1.99	50.23^{b} ±2.48	0.93^{ab} ±0.04	1.18^{a} ±0.05
3	9.07 ±0.27	4.35^{a} ±0.09	4.72 ±0.18	66.08 ±2.13	70.00^{a} ±8.81	1.20^{a} ±0.14	1.23^{a} ±0.04
4	8.35 ±0.81	4.12^{b} ±0.03	4.23 ±0.79	66.65 ±5.97	55.62^{ab} ±1.29	1.12^{a} ±0.17	1.22^{a} ±0.09

TP: total protein, g/dl; AL: albumin, g/dl; GL: globulin, g/dl; AST: aspartate aminotransferase, u/dl; ALT: alanine aminotransferase, u/dl; Crea.: creatinine, mg/dl; U. acid, uric acid, mg/dl. a-c: means in the same column superscripted with different letters, significantly (P≤0.05) differ.

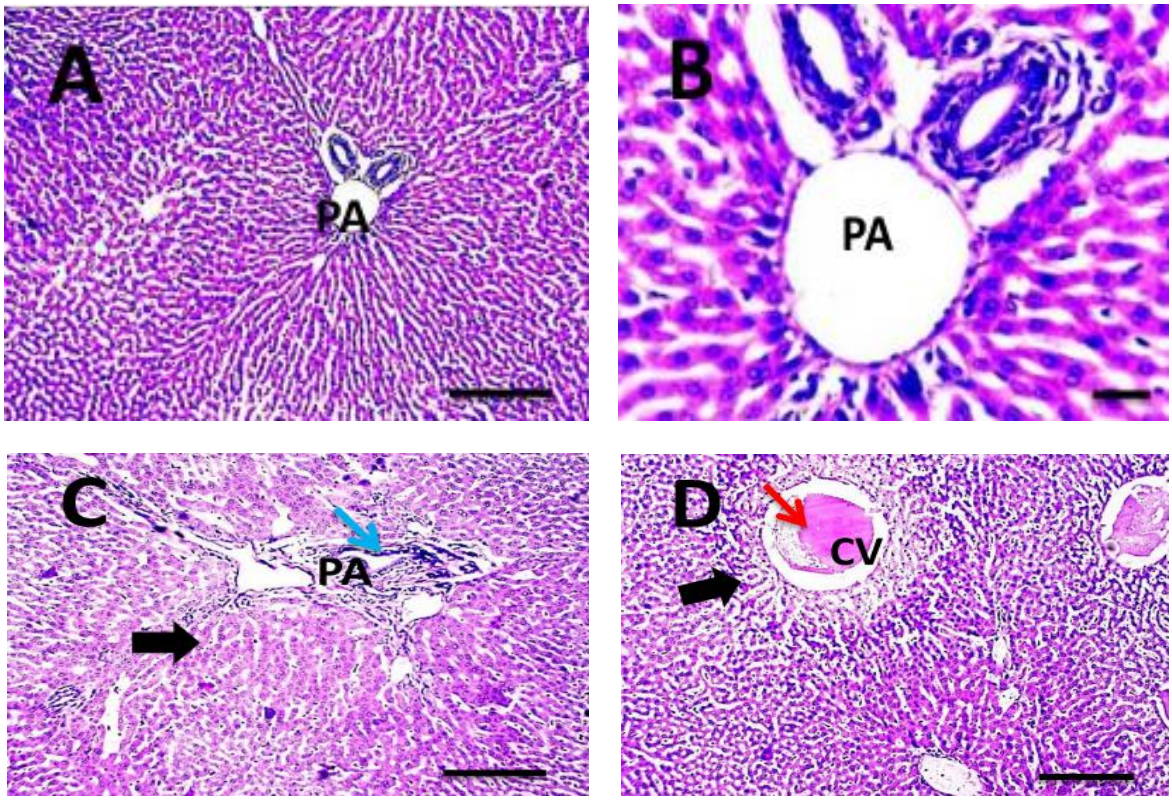
Table 7. Effect of the dietary treatments on the experimental rabbits' plasma glucose and total lipids (mean ± standard error), at the end of the entire experimental period

Groups	Glu.	Cho.	Trig.	HDL	LDL
1	98.85 ±2.02	100.40^{a} ±9.23	61.02^{a} ±8.64	59.08^{ab} ±1.60	29.10 ±9.34
2	98.18 ±2.98	95.58^{b} ±5.35	53.53^{b} ±0.43	63.58^{a} ±2.58	21.30 ±5.71
3	96.28 ±4.54	79.42^{d} ±3.31	55.75^{b} ±4.89	52.15^{b} ±3.83	16.12 ±3.53
4	95.47 ±3.42	84.25^{c} ±12.82	56.62^{b} ±7.79	47.02^{c} ±3.87	25.93 ±9.53

Glu.: glucose, mg/dl; Cho.: cholesterol, mg/dl; Trig.: triglycerides, mg/dl; HDL: high density lipoprotein, mg/dl; LDL: low density lipoprotene, mg/dl. a-d: means in the same column superscripted with different letters, significantly (P≤0.05) differ.

3.12. Histology of liver and kidney

Figures 6 and 7 illustrate the histological alterations occurred in liver tissue from the PE-treated rabbits and their statistical analysis, respectively. Whereas Figs. 8 and 9 present those of the kidney tissue.



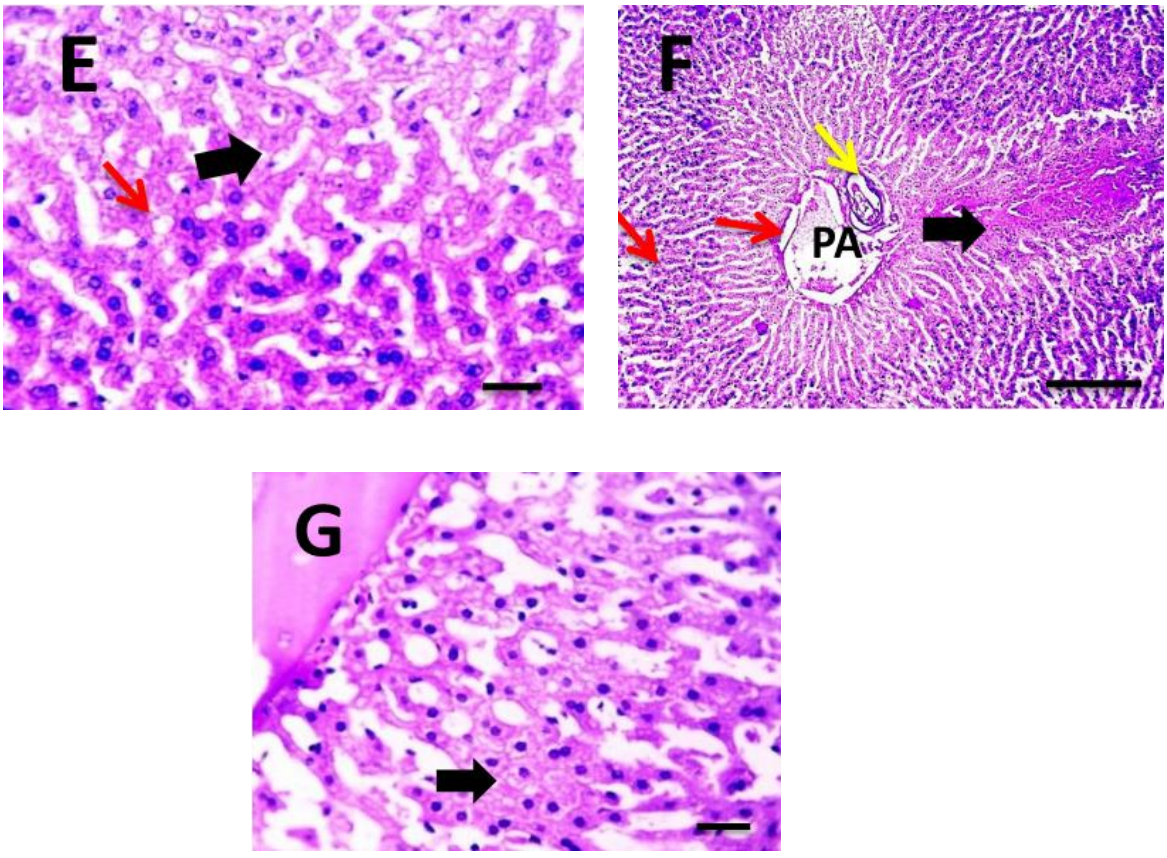


Fig. 6. Microscopic pictures of H&E stained liver sections showing normally organized hepatic cords, central veins (CV) and portal areas (PA) in control negative group (A&B), disorganization of hepatic cords (thick arrow), dilation of bile duct with biliary hyperplasia (blue arrow) in group received 0.1 polyethylene (C), congestion of portal vein (red arrow), centrilobular necrosis of hepatocytes (thick arrow), in group received 0.2 polyethylene (D&E), congested portal blood vessels (red arrow), peri-portal necrosis of hepatocytes (thick arrow) and cholestasis (yellow arrow) in group received 0.4 polyethylene (F&G). (A,C,D,F) X: 100 bar 100 (B,E,G): X: 400 bar 50.

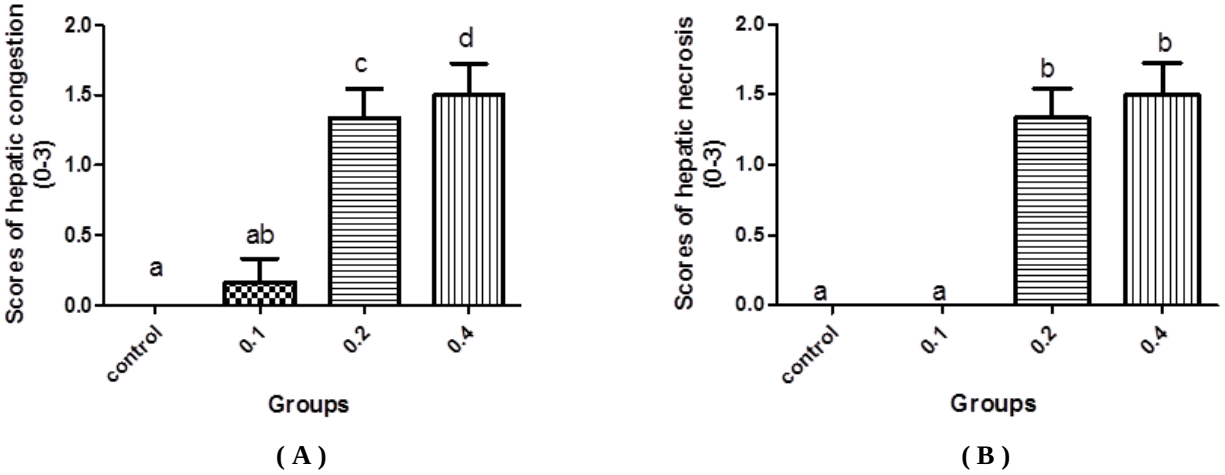


Fig. 7. Statistical analysis shows significant increase of scores of hepatic congestion (A) and necrosis (B) in group received 0.4 polyethylene when compared with control group. Different small alphabetical letters means significant (when $P<0.05$).

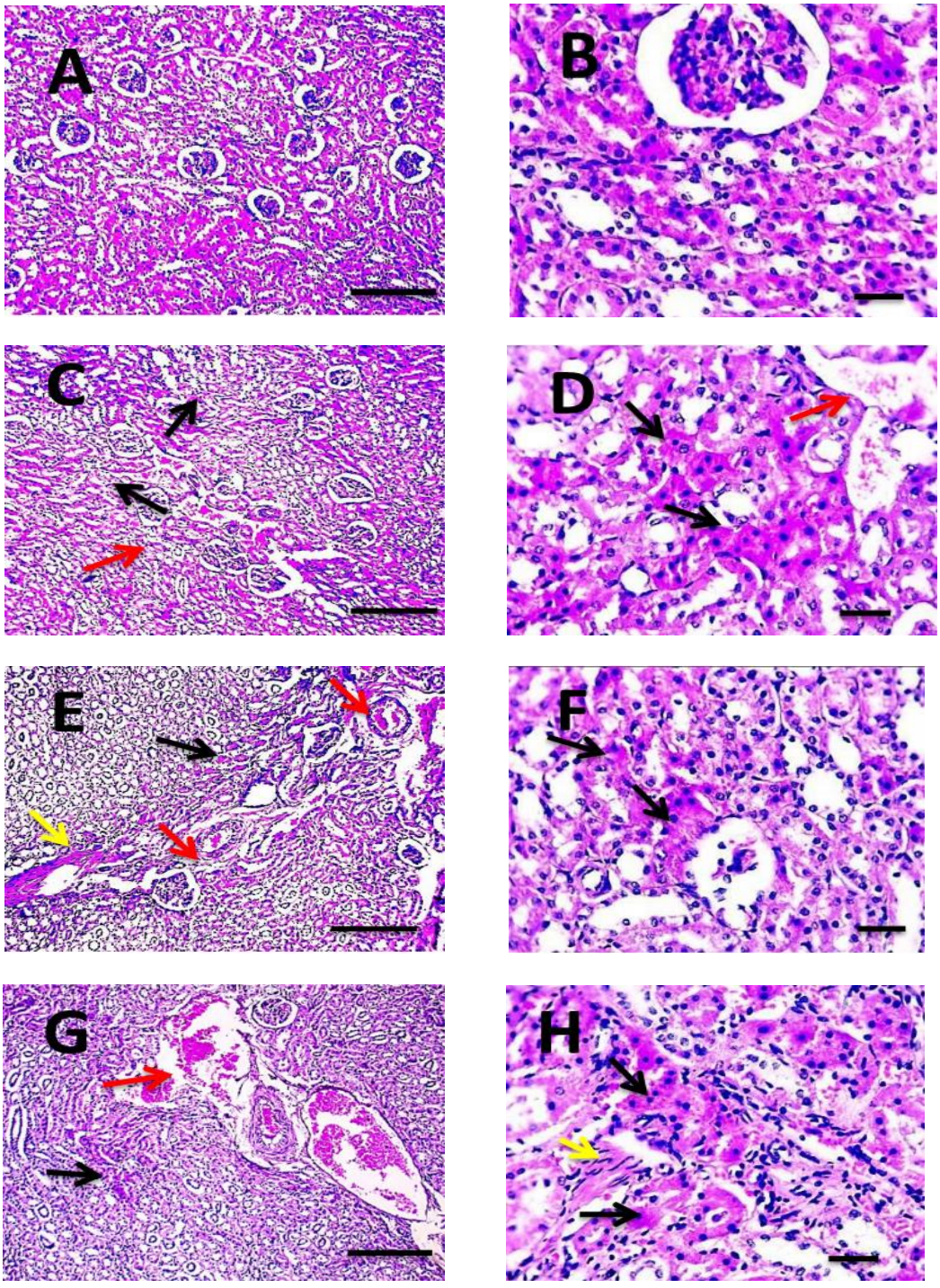


Fig. 8. Microscopic pictures of H&E stained kidney sections showing normally glomeruli, renal tubules and interstitial tissue in control negative group (A & B), mild congestion (red arrow), mild acute tubular necrosis (black arrows) in group received 0.1 polyethylene (C & D), mild congestion (red arrow), mild interstitial fibrosis (yellow arrow) and mild tubular necrosis (black arrows) in group received 0.2 polyethylene (E&F), moderate congestion (red arrow), mild interstitial fibrosis (yellow arrow) and moderate tubular necrosis (black arrows) in group received 0.4 polyethylene (G&H). (A, C, E, G) X: 100 bar 100 (B, D, F, H): X: 400 bar 50.

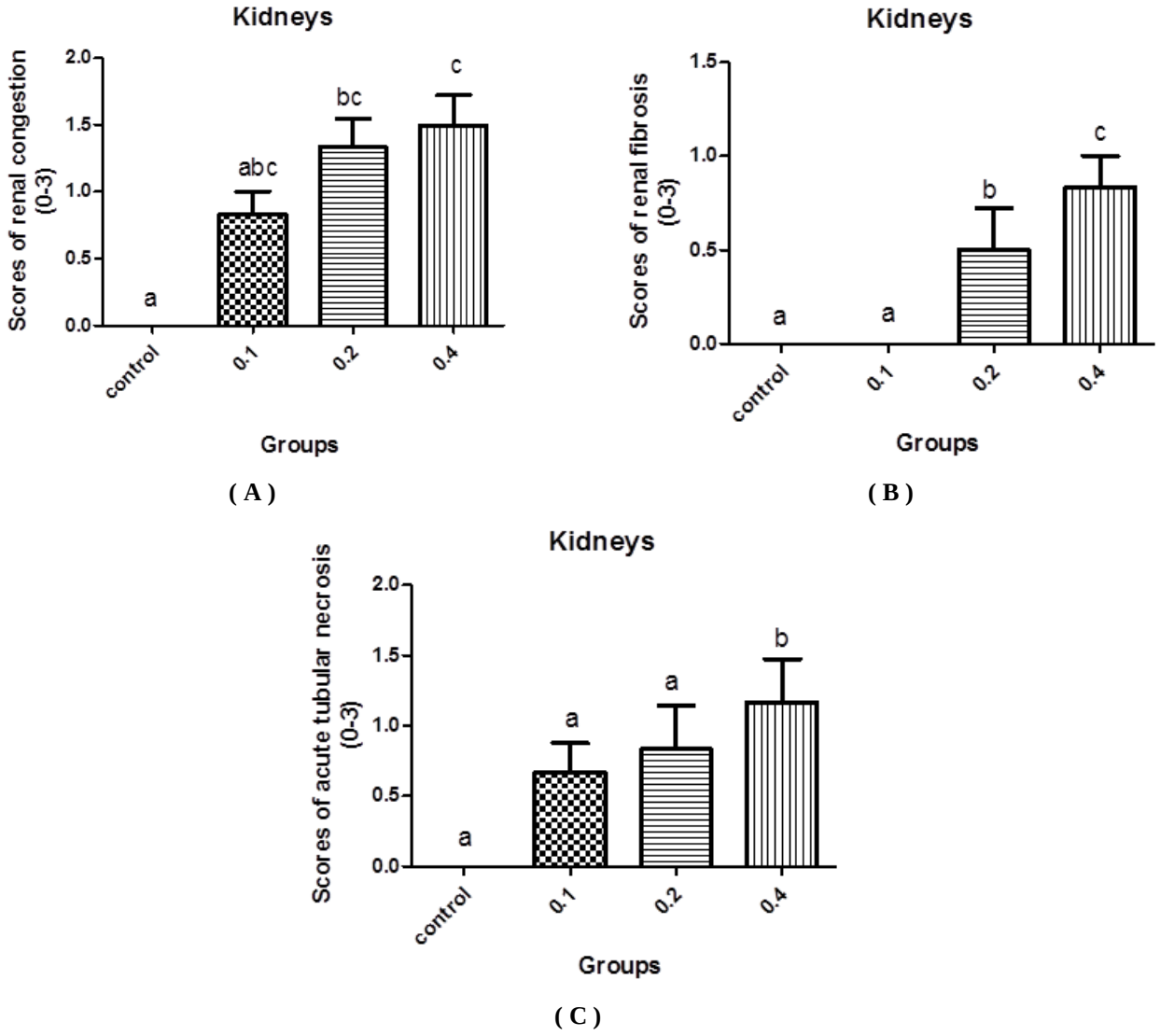


Fig. 9. Statistical analysis shows significant increase of scores of renal congestion (A), fibrosis (B) and acute tubular necrosis (C) in group received 0.4 polyethylene when compared with control group. Different small alphabetical letters means significant (when $P<0.05$).

4. Discussion and Conclusion

Polyethylene is an outspread environmental pollutant. It could be used too in manufacturing the pelleted animal feeds. From the foregoing results in the present study, its presence in the rabbits' diets caused different significant negative changes included clinical and post-mortem symptoms, disorders of digestion and absorption led to bad feed utilization, low specific gravity of bone, fewer carcass quality besides PE-residues, as well as changes in blood picture and histological alterations of both liver and kidney. Higgins [20] applied a new technique for polyethylene determination using film FTIR calibration techniques (versatile Agilent Cary 630 FTIR spectrometer).

Rearing animals under similar contaminated conditions caused also negative effects. So, Abdelhamid *et al*. [21] obtained results revealed the negative effects of feeding rabbits the dioxin (a derivative of polyethylene)-contaminated diets that were responsible for very bad appearance of the face, nose and eyes, stench smell (similar to that of the dioxane itself) of the rabbits and their feces as well as their flesh (during the chemical analysis of the flesh), moderate vertebrates cord malformation, smaller size and weight, weakness and unbalance, increased food and

water consumption followed by lower food conversion and economic efficiency as well as boneless meat and protein contents of the carcasses. Hence, the avoidance of such contaminant, in foods and drinking water, must be recognized for good rearing of the very sensitive animals such as rabbits. Abdelhamid *et al*. [22] added that the dietary inclusion of 1,4-dioxane was responsible for occurring a case of multiple anemia [aplastic (toxic), leukemia toxicity, and malnutrition] besides toxic hepatic and biliary tract disease, dehydration, steatorrhea, edema, myocardial infarction and/or excessive protein catabolism. Hlatini and Chimonyo [23] found that the presence of polyphenolic compounds limits the utilization of leaf meals. So, pigs fed the *Acacia tortilis* leaf meal treated with polyethylene glycol (PEG) showed a linear response on total protein (TP) and globulin, but quadratic to albumin ($P \leq 0.01$). There was a linear relationship between PEG inclusion and cholesterol, creatinine and uric acid ($P \leq 0.05$). The activity of aspartate aminotransferase ($P \leq 0.01$) and alanine aminotransferase ($P \leq 0.05$) decreased linearly as PEG inclusion increased. There was a quadratic increase in alkaline phosphatase as the PEG inclusion level increased ($P \leq 0.05$). In addition, Abdelhamid *et al.* [24] concluded that propylene glycol increased ($P \leq 0.05$) the feed intake and live body weight, compared with the control. The treatment significantly ($P \leq 0.05$) reduced blood urea nitrogen, and non-essential fatty acids' values; but increased blood insulin, T3, and T4, concentrations and T3/T4 ratio.

Heavy metals [25] and mycotoxins are among the toxicants affect not only rabbits' [26]-[28] and rats' [29]-[31] performance, feed utilization, and internal organs' functions and structures, a; but also human health [32]. So, other toxicants could be the causative of similar disorders found herein. For example, Tang *et al*. [33] studied the effect of aflatoxin (AF) exposure with a group of Ghanaian participants. AFB1–albumin adducts (AFB-AA) were measured. A significantly negative correlation was found between serum AFB-AA and vitamin A levels. An even stronger, significant negative, correlation was found between serum AFB-AA and vitamin E levels. Serum AFB-AA levels were statistically higher in subjects who had low levels of both vitamins A and E. Significantly negative correlations were confirmed between levels of serum AFB-AA and both vitamins A and E. Again, high serum AFB-AA concentrations were found in subjects with low levels of vitamins A and E compared with the concentrations in subjects with high levels of vitamins A and E. These data show that AF exposure was associated with decreased levels of serum vitamins A and E in high-risk human populations, which may significantly influence the incidence of AF-related adverse health effects.

Jollya *et al.* [34] also examined the association between certain clinical factors and aflatoxin B1–albumin adduct (AF-ALB) levels in HIV-positive people. Multivariable logistic regression showed statistically significant increased odds of having higher HIV viral loads and higher direct bilirubin levels among HIV-positive participants in the high AF-ALB group. There were also higher levels of total bilirubin and lower levels of albumin in association with high AF-ALB. Thus, aflatoxin exposure may contribute to high viral loads and abnormal liver function in HIV-positive people and so promote disease progression. Hasanzadeh and Amani [35] detected in female healthy adult Wistar rats received graded levels of aflatoxin B1 (AFB1) via gavage for a period of 25 days, an increase in the concentration of AFB1 resulted in a reduction in the population of healthy primordial, primary, secondary and tertiary follicles. In both the right and left ovaries, all types of atretic follicles, including primordial, primary, secondary, and tertiary atretic follicles were significantly increased ($P<0.01$). In conclusion, AFB1 is gametotoxic for all type of ovarian follicles, and the atretogenic effect of AFB1 is dose dependant. Abdallah *et al*. [36] alarmed that the contamination rates in the investigated regions for AFB1 in maize were dander.

Hassena *et al.* [37] confirmed that zearalenone (ZEN) induces Hsp 70 expression in a time and dose-dependent manner; it could be therefore considered as a biomarker of toxicity. A cytoprotective effect of Hsp 70 was elicited when Hep G2 cells were exposed to sub-lethal heat shock prior to ZEN treatment and evidenced by a reduced ZEN cytolethality. Data showed that ZEN is cytotoxic in Hep G2 cells by inhibiting cell proliferation and total protein synthesis and pointed out oxidative damage as possible pathway involved in ZEN toxicity. Also, Obremski *et al*. [38] studied the histopathology lesions in the internal organs of hyplus line rabbits suspected of having zearalenone (ZEA) intoxication. The results obtained confirmed the presence of ZEA in both the blood and liver. The histopathology pattern of the uterus and ovaries were characterized by enlarged uterine glands, softening, oedema in the tunica mucosa and destroyed granule layer of the ovarian follicles. Thus the disturbances noted in the reproductive system of the rabbits were caused by the intake of feed containing zearalenone. Moreover, Alexander *et al.* [39] review the opinion of the European Food Safety Authority belonging to the European Commission concerning the risk to consumers of a possible increase of the maximum level (ML) for zearalenone in breakfast cereals. The highest concentrations of zearalenone were reported for wheat bran, corn and products thereof (e.g. corn flour, cornflakes).

Grains and grain-based foods, in particular grains and grain milling products, bread and fine bakery wares, made the largest contribution to the estimated zearalenone exposures. Vegetable oils also made an important contribution to the zearalenone exposure. The critical effects of zearalenone result from its oestrogenic activity. Also, Tsouloufi *et al*. [40] confirmed that ZEN affected some of the clinicopathologic syptoms of adult rabbit bucks; these changes were mostly indicative of mild hepatocellular damage and dysfunction, inflammatory and/or allergic responses, as well as renal tubular damage.

Abdelhamid *et al*. [41] in a feeding experiment with rats to reevaluate the possible toxic effects of very low level (tolerance limit) of ochratoxin A (OTA) revealed that the toxicated groups of rats moisten their mats than the other groups' mats. All toxic diets reflected high bone mineral concentration. Bone area was increased in all treatments. Lean mass was the lowest in the toxic diets. Fat mass was at lowest value in the toxic diet. OTA affected also the post-mortem, relative weights (particularly spleen), the blood profile (particularly Glob, Glu, AST, Cre, alkaline phosphatase, LDH, testosterone, lymphocytes, monocytes, and granulocytes), proximate analysis of the biological tissues, and the histological structure of the internal organs. So, even the very low concentration of OTA (25 ppb, as a dietary tolerance level in some countries) used in this study may be harmful. So, it is still a fact that prevention (of mold growth) is easier, cheaper and more effective and better than curing of mycotoxicosis.

Suriamurthy and Srikumar [42] registered too differential response to the gibberellic acid (GA3) doses offered to rats. Levels of cholesterol, triglyceride, protein, albumin, urea, uric acid and creatinine were augmented with that of malondialdehyde (MDA) and glutathione peroxidise (GPx) at all doses of GA3 used. In contrast, the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) level reduced significantly. Deoxyribonucleic acid (DNA) content decreased. Results indicated that GA3 induced oxidative damage in rat testicular cells and augmented transamination, dephosphorylation and amylolytic activities. GA3 decreased the cellular antioxidant defence potential as also the DNA content. Spectrums of histological aberrations were noted due to the GA3 effect. GA3 caused testicular toxicity through peroxidative damage and raised cellular metabolic activity. GA3 suppressed testicular antioxidant catalytic potential and promoted DNA damage. Moreover, Öztürk *et al*. [43] conclude too that gibberellic acid influenced the cytokine balance. The resulting changes may be responsible for the development or advancement of inflammatory diseases and malignancies in rats.

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