RESEARCH ARTICLE

Physiological, Histological, and Biochemical Evidence for the Effect of Two Azo Dyes on Mice Model

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ABSTRACT

The effects of (*E*)-*N*-(4-hydroxy-3-(((4-(5-(p-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl) phenyl) sulfonyl) diazenyl) phenyl) acetamide (L1) and (1*S*,2*R*)-3-(isopropyl amino)-1-(4-(2-methoxy ethyl) phenoxy)-1-((*E*)-(3-nitrophenyl) diazenyl) propan-2-ol (L2), in the *Mus musculus* L. from Balb/C were studied. These azo dyes showed well ability to destroy cancer living cells and reduce the growth of human breast MDA-MB231 cancer cells. Therefore, the complete blood count (CBC) was then done for the three groups of mice A (injected with L1), B (injected with L2) and C (the control). The results were indicated that the changes in the red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), blood platelets (PLT) and the white blood cell count (WBC), were very slightly effected by each azo dye in contrast with the control. The average of data received by each test then was calculated. The results of WBC were displayed a little bet lemphobinia, which can be improved using vitamins. Also, the statistical analysis was performed by using SPSS version 20 with $p < 0.05$ at a significant data, which expressed according to Mann-Whitney test, Kruskal-Wallis test, and multivariate ANOVA. Further, the cutoff values for each blood test were also intended. The results were revealed that the cutoff values were variable and seems to be reasonable in contrast with the control. These results were confirmed by using the tissues of the investigated organs (heart, kidney, and liver). The results were looked acceptable because, the mice are still alive and are active until diesection. Hence it is recommended that these azo dyes as a new anticancer drugs.

Keywords: Azo dyes, Hematocrit, Hemoglobin, Lemphobinia, Mean corpuscular hemoglobin, Red blood cell.

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INTRODUCTION

Azo compounds were receiving high attention in scientific research,[1-4] and they have great importance in chemical analysis. A strongly colored compound can be yellow, red, orange, blue or even green, depending on the exact structure of the molecule. It makes azo dyes as extremely important as dyes and as pigments for a long time.[5] The structural features in the organic compounds that usually produce color are C=C, N=O, N=N, aromatic rings, C=O, and NO_2. Though the groups that invariably confer color are the azo (–N=N–) and nitroso (–N=O), while the other groups actually do so under certain circumstances.[6] Azo dyes contain one or more azo groups (–N=N–) which are linked to SP_2 hybridized carbon atoms, based on the number of such groups.[7] These compounds contain more than one active group, which is able to formulate chelatic coordinational complexes with metal ions distinguished by their color and ability to dissolve in different solvents.[6] Further, the azo is reactive compound,[5] that was reported for its pharmaceutical importance as antidiabetic,[8] antineoplastic,[9] antibacterial,[5,10] and anticancer agent.[11]Add to this, the azo molecules are known to be involved in the inhibition of DNA, RNA, carcinogenesis, and protein synthesis. The presence of -N=N- in the molecular structure of azo is responsible for the interaction with the active site of a protein.[11] The reduction of azo dyes, i.e., the cleavage of the azo linkage (s), leads to the formation of aromatic amines that are known as mutagens and carcinogens.[12] In mammals, metabolic reduction of food azo dyes is mainly attributable to bacterial activity in the lower gastrointestinal tract's anaerobic parts.[12] Various other organs, especially the liver and the kidneys, can also reduce food azo

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dyes. After degradation in the intestinal tract, the released aromatic amines are absorbed by the intestine and are then excreted via the kidneys. Azo dyes are generally recalcitrant to biodegradation because of their complex structures, but some microbial consortia or combinations of anaerobic and aerobic systems achieve complete degradation.[12] Lactic acid bacteria (LAB) as promising probiotic isolates could completely metabolize some azo dyes under anaerobic/aerobic regimes. Probiotics are health-promoting live microorganisms that improve the intestinal microbial balance and produce various compounds that inhibit the growth of various bacterial pathogens.[12]

MATERIALS AND METHODS

Laboratory Animals

In the current study, the experimental animals were mice *Mus musculus* L. from Balb/C, which prepared from the Department of Pharmaceutical Control of Baghdad Governorate and raised in the Animal House of the Department of Life Sciences, College of Education for Pure Sciences, Basrah University under similar conditions, 20–25°C and a 12 hours fixed lighting system 12 hours light.[13] The mice were placed in plastic cages of standard size (30 × 12 × 11 cm). The floor of the cages was sprayed with wood shavings, which are replaced weekly, and the animals were fed in a specific diet. The laboratory mice were 10–12 weeks and 23–25 g divided into three groups contain five mice in each. Group C served as a control group; injected with 0.1 mL of normal saline, group A; injected with 0.1 mL of L1 of 0.002 micromolari/ kg concentration, and group B injected with 0.1 mL of L2 of 0.0005 micromolari/ kg, one injection for 15 days and then we kill the animals for laboratory tests.

Physiological Tests and Histological Sections

Draw blood from the heart immediately after anesthesia of male mice with chloroform using a 1-mL syringe and put them in special tubes containing EDTA, which prevents clotting,[13] to measure some hematological parameters (RBC, WBC, lymphocytes%, monocytes%, granularcyte%, HGB, Hct, MCV, MCH, MCHC, PLT) while the organs (heart, kidney, liver, testis) were removed and stored in special stabilizers.

RESULTS AND DISCUSSION

The pharmaceutical azo dyes,[14] that named (*E*)-*N*-(4-hydroxy-3-(((4-(5-(p-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl) phenyl) sulfonyl) diazenyl) phenyl) acetamide (L1) and (1*S*,2*R*)-3-(isopropyl amino)-1-(4-(2-methoxy ethyl) phenoxy)-1-((*E*)-(3-nitrophenyl) diazenyl) propan-2-ol (L2) were synthesized, (Figure 1). and then were characterized using m.p., IR, UV-visible and ^{1}H NMR spectrum respectively.[14] The synthetic azo dyes were provided non-toxic effects using different concentrations from each and didn't show any hemolysis effect in the cells. The cell viability (cytotoxicity assay),[14] is used for each dye to observe their ability in destroying cancer-living cells. And reduce its growth for human breast MDA-MB231 cancer cells after 24 hours treatment with 100 µM of each dye. The results were showed well activities of each dye against cell viability in contrast with the control. Further, the results of human DNA binding of each azo dye were indicated its ability to damage DNA and inhibiting of DNA transcription and replication. These results with that obtained by the NanoDrop™ spectrophotometer were displayed increasing in the concentration of the nitrogen bases,[14] which confirmed that the DNA was damaged. Due to recommend the synthetic, non-toxic azo dyes as novel drugs for the treatment of human breast MDA-MB231 cancer cells through its ability to destroy the cancer cells' DNA.[14]

Therefore, the effect of the L1 and L2, (Figure 1) in the *Mus musculus* L. from Balb/C were studied using three groups of *Mus musculus* L. from Balb/C that fed a specific diet until age 10–12 weeks with weight 23–25 g. the groups were divided into five mice of each. Group A, group B, and group C as mentioned above. the CBC test, (RBC, WBC, Lymphocyte%, Monocyte%, Granularcyte%, HGB, Hct, MCV, MCH, MCHC. PLT) of each group were done, and the average of the data from each blood test was also calculated (Figure 2). These blood tests were verified for mice groups A and B that were injected with each of L1 and L2, respectively. These results were then compared with that received by the mice injected with control (group C). further, the statistical analysis was performed using SPSS version 20 with $p < 0.05$ at a significant data are expressed according to Mann-Whitney test, Kruskal-Wallis test, and multivariate ANOVA.

Figure 2 above shows that the CBC and blood biochemistry were seeming to be normal in contrast with the control. The

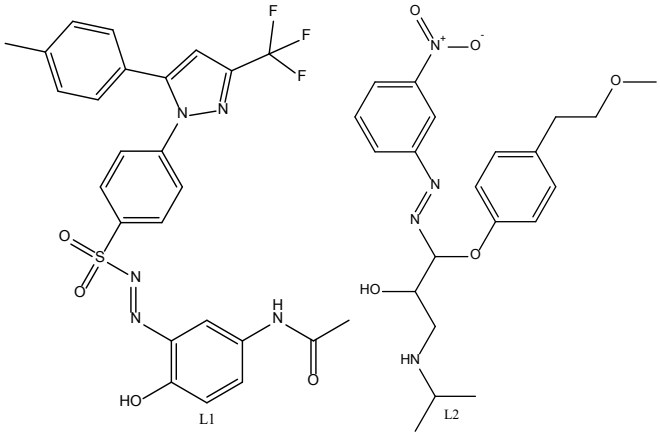

Figure 1: The structures of each azo dye.[14]

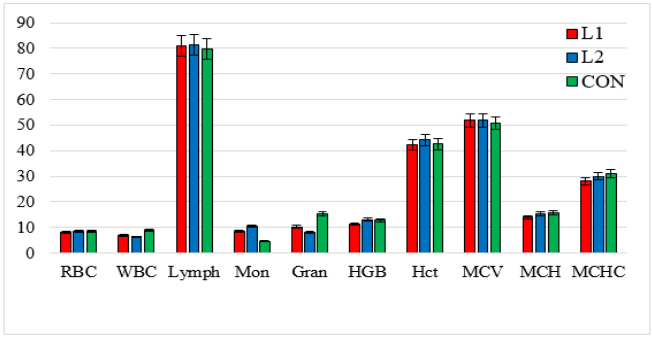

Figure 2: The average of data of each blood test.

Table 1: The cutoff values for each blood test performed on mice injected with each azo dye (L1 and L2) solution in contrast with the control

Id	*RBC*	*WBC*	*Lymph (%)*	*Mon (%)*	*Gran (%)*	*HGB*	*HCT*	*MCV*	*MCH*	*MCHC*	*PLT*
L1	8.16	6.87	80.8	8.67	10.32	11.35	42.3	51.8	13.9	28.1	996.6
	0.49	0.81	1.7	0.88	0.93+	0.99	2.5	1.2	0.91	1.3+	103.6
L2	8.52	6.32	81.28	10.48	8.24	13.12	44.15	51.9	15.4	30	1094.6
	0.4	0.6	2.1	1.15	1.02	0.6	2.09	0.63	0.31	0.36+	95.5+
control	8.63	9.06	79.9	4.56	15.52	12.89	42.6	50.8	15.7	31.2	1156.7
	0.28+	0.67	3.5	0.44	3.6	0.38+	1.08	1.74	0.2	1.3+	68.1+

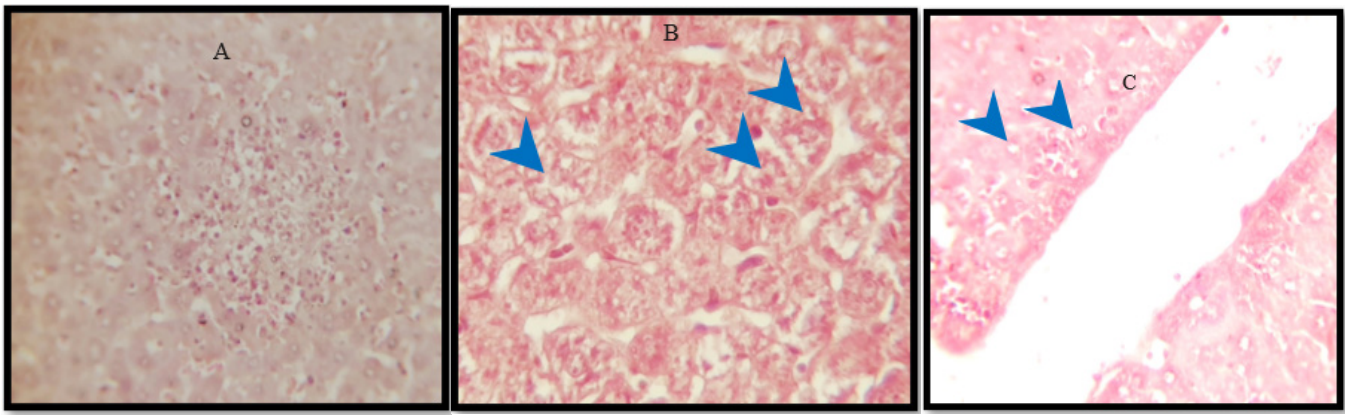

Figure 3: Sections of liver, group A (L1), group B (L2) and group C (the control).

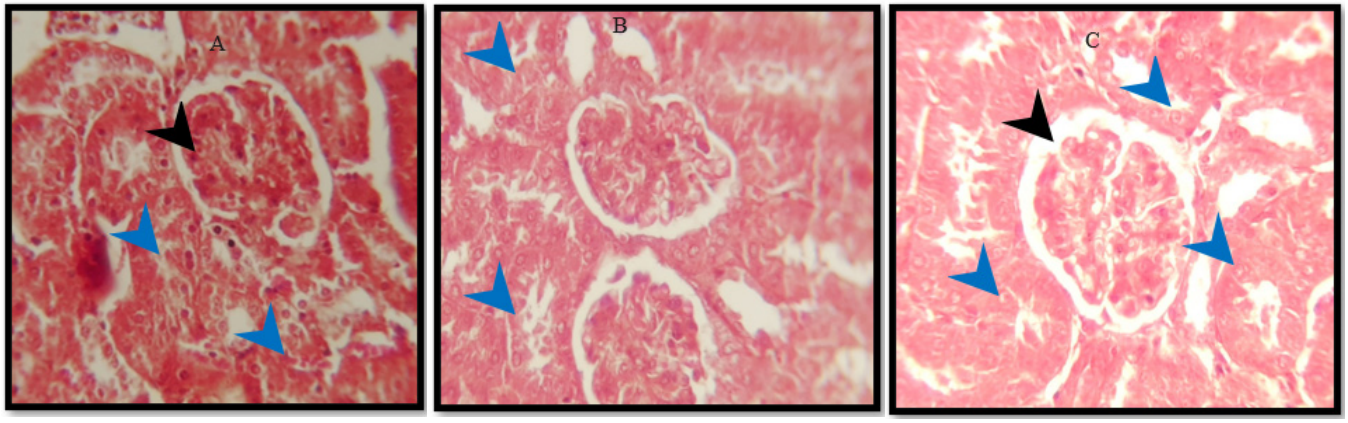

Figure 4: section of kidney, group A (L1), group B (L2) and group C (the control).

changes in the RBC, HGB, HCT, MCV, MCH, MCHC, PLT and WBC were very slightly detected in the group's A and B of mice, that were injected with each azo dye L1 and L2, respectively in contrast with the control. The WBC were also showed a little bet lymphopenia, which can have improved using vitamins.[15] Add to which the cutoff values for each blood test as presented in Table 1 below were intended for each group (A and B). The results were then compared with those received by the control group C.

Table 1 above displays that the cutoff values received by the RBC, HGB, HCT, MCV, MCH, MCHC, PLT and WBC were variable and seems to be reasonable in groups A and B in contrast with the control C. These results were confirmed using the tissues of the investigated organs (heart, kidney and liver), (Figures 3 to 5) below.

The figures display that the liver tissues of the investigated organs from groups A, B and C were showed that the effect of L1 and L2 were (normal hepatocytes in both cerntri-lobular area and periportal area, congested hepatic vasculature, inflammation in the hepatic parenchyma, normal hepatocytes in the cerntri-lobular area, congested hepatic vasculature and congestion of central vein) and (vacuolated hepatocytes in the centri-lobuar area and vacuolated hepatocytes in the centrilobular area) respectively. These results were then compared with the control group received, which showed normal hepatocytes in both the cerntri-lobular area and periportal area, normal hepatocytes in the periportal area, and normal hepatocytes in the cerntri-lobular area. However, the kidney tissues of the investigated organs from groups A, B and C were showed that the effect of L1 and L2 were normal glomerulus and renal tubular epithelium in the renal cortex and vacuolated renal tubular epithelium in the renal medulla and hemorrhage in the renal pelvis and mild degeneration of renal tubular epithelial cells and mild degeneration of renal

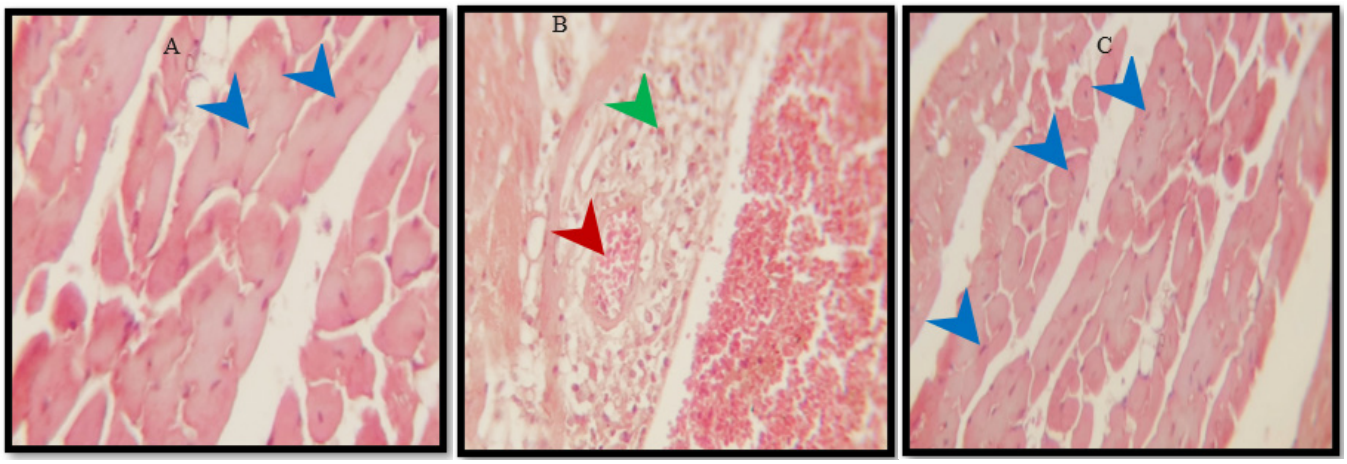

Figure 5: section of heart, group A (L1), group B (L2) and group C (the control).

tubular epithelial cells respectively in contrast with the control normal glomeruli and normal renal tubules in the renal cortex, normal glomerulus and normal renal tubules in the renal cortex and normal renal tubules in the renal medulla. Also, the heart tissues of the investigated organs from groups A, B, and C were showed that the effect of L1 and L2 were (normal myocardial muscle fibers and normal myocardial muscle fibers) and (vacuolated myocardial muscle fibers, vascular congestion, perivascular inflammation, vascular congestion, and perivascular inflammation) respectively in contrast with the control (normal myocardial muscle fibers and normal myocardial muscle fibers). The results were looked acceptable because the mice are still alive and are active until dissection.

CONCLUSION

The synthetic azo dyes (L1 and L2) that synthesized cheaply gained good color delivered non-toxic effects; they didn't show any hemolysis effect in the cells. Beside its ability to bind breast cancer MDA-MB231 cells and affect the cell viability%, also their ability to damage DNA and inhibit DNA transcription and replication. The synthetic azo dyes can also be affected the CBC test using a group of mice injected with each. The injected mice are still alive and are active until dissection. Statistical analysis was performed by using SPSS version 20 with $p < 0.05$ at a significant data. Further, the results of the average and the cutoff values were revealed variable and reasonable. These results were confirmed by means of the tissues of the investigated organs (heart, kidney, and liver), which were observed suitable—owing to recommend these azo dyes as new anticancer drugs.

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