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Toxicity Profile Study of Antihypertensive Drug Prazosin in Pregnant Wistar Rats

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Abstract

The purpose of this research is to investigate and assess the cytotoxicity and genotoxicity of Prazosin HCL in pregnant rats. Prazosin (PZ) was administered to the animals intraperitoneally (IP) at dosages of 5, 15, and 25 mg/kg/body weight for single dose (14-day) toxicity tests. The following parameters have been examined for evaluating genotoxicity: bone marrow micronucleus assay, peripheral blood micronucleus assay, DNA damage is measured using the metaphase chromosomal analysis, DNA damage is measured using the DNA fragmentation test, and cytotoxicity is measured using a histological analysis. The results obtained clearly demonstrate that PZ induced hazardous responses at the higher dose in the hepatocytes, as evidenced by DNA damage, and increased DNA fragmentation in pregnant rats. Observing that PZ significantly increased DNA strand breakage and structural chromosomal aberrations in bone marrow cell lines is also fascinating. As a result, it is thought to be genotoxic to bone marrow and mouse hepatocyte cells. The current study showed that Prazosin had significant genotoxic effects in pregnant rats at the matching hepatotoxic dose level.

Keywords: *Genotoxicity, Cytotoxicity, pregnant rats, histology, micronucleus, blood pressure, Prazosin.*

1. Introduction

The increase in blood pressure in the body is known to exert effect on the arteries of the body. This medical condition persisting in the body is known as hypertension. The negative effect exerted as a result of high blood pressure is increased pressure at a constant rate against the walls of the artery. As a result, the heart requires to work with extra efforts in order to pump blood [1]. The units used commonly for the measurement of blood pressure are millimetres of mercury or mm Hg. The medical condition 'hypertension' refers to increased blood pressure of more than 130/80 mm Hg. The class of hypertension has been divided into four major categories by the American Heart Association and the American College of Cardiology. For classifying all types of blood pressure, normal blood pressure is considered to be ideal [2].

In 14 patients with essential hypertension, the mechanism of action of the novel antihypertensive drug Prazosin hydrochloride was investigated. After eight weeks of therapy, the group's mean supine blood pressure decreased from 144/99 +/- 2/1 (SE) mm Hg at baseline to 140/92 +/- 4/3 ($P<0.05$). The individuals who responded to treatment did not exhibit any substantial postural hypotension [3]. Some of the parameters like the rate of glomerular filtration, effective flow of plasma through renal route, and stimulated peripheral plasma renin activity were reported to not change throughout the therapy [4].

Although plasma volume increased in fifteen of the twenty patients in whom it was assessed, cardiac output did not alter significantly (P<0.025). Peripheral vascular resistance decreased significantly (P less than 0.025) among the patients whose mean blood pressure dropped by at least 10 mm Hg, although the reduction in plasma volume was not statistically significant [5]. Peripheral vascular resistance did not alter substantially among the individuals who saw a mean blood pressure drop of less 10 mm Hg as a result of treatment, but plasma volume did (P less than 0.025). A potent antihypertensive drug that works by peripheral vasodilatation is Prazosin hydrochloride. It could result in fluid retention [6]. The medication doesn't seem to have an impact on renin secretion or renal function.

In a single-blind comparison investigation of the cases of 30 moderately hypertensive patients, the blood pressure-lowering effects of clonidine hydrochloride and Prazosin hydrochloride were comparable. The renin-aldosterone axis was not significantly affected by either substance. The antihypertensive effects of Prazosin and clonidine were both noticeably enhanced by the addition of polythiazide and chlorthalidone, respectively [7]. When Prazosin and clonidine were administered, serum cholesterol levels were seen to fall; while the same levels increased as a result of addition of the diuretics to the regimen. The side effects of clonidine, especially sleepiness and dry mouth, were problematic for the individuals who received it. Prazosin was well-tolerated, and its adverse effects tended to lessen over time. Two patients who received Prazosin experienced the "first-dose" effect, but treatment was unaffected. Significant hypokalaemia was caused by both diuretics [8].

Experimental animals

The Institutional Animal Ethics Committee (IAEC) gave its approval to each and every animal experiment. Male Swiss albino pregnant rats were purchased from the Central Animal Facility of the Institute for the experiments. The study employed 7-week-old Swiss albino pregnant pregnant rats that weighed 25–30 g. These animals were kept in controlled environments with alternate 12 hour light and dark cycles, room temperature (22.2°C), humidity (50%), and other environmental factors. A commercial provider provided the standard laboratory animal diets, and the animals also received libitum water. The animals spent a minimum of two to three days becoming acclimated to the test environments before dosing began.

Chemicals

All of the reagents and chemicals utilized in the investigation were of the analytical grade.

Drugs

Cyclophosphamide was bought from Hi-Media, and prazosin from Sigma Aldrich. Colchicine, diphenylamine, foetal bovine serum (FBS), thiobarbituric acid (TBA), Ellman's solution (5, 5-dithiobis-2-nitrobenzoic acid), sulfosalicylic acid (5%), and bovine serum albumin (5%). These substances were all bought from Sigma Aldrich.

Decision Making in Dose Selection

The trials on animals and humans done in the past formed the basis of selection of dose in the present study. Therefore, the doses selected for the study were 5, 15 and 25 mg/kg.

Therapy Protocol

The animals were divided into five groups consisting of six healthy pregnant rats in each group for the objective of evaluating cytotoxicity and genotoxicity investigations in Swiss albino adult healthy pregnant pregnant rats of the drug Prazosin.

1. Deionized water (2 ml/kg; i.p.) was given to Group 1 (Normal Control) once every day for 14 days.
2. On the 12th day of the 14-day research, Cyclophosphamide (30 mg/kg; i.p.) diluted in distilled water was administered to Group 2 (Standard).
3. Prazosin (5 mg/kg; i.p.) was given on daily basis till the completion of 14 days to Group 3 (PZ) in de-ionized water after slight heating.
4. Prazosin (15 mg/kg; i.p.) was given on daily basis till the completion of 14 days to Group 4 (PZ) in de-ionized water that had been slightly heated.
5. Prazosin (25 mg/kg; i.p.) was given on daily basis till the completion of 14 days to Group 5 (PZ) in de-ionized water after mild heating.

Evaluation of Cytotoxicity: Micronucleus Bone Marrow Assay

Diethyl ether was inhaled to put the animals to sleep, and then the diaphragm was cut. Six surviving animals in the treatment and control groups had their femurs amputated in order to collect their bone marrow. The marrow that was removed from the bones of each animal was mixed in a single centrifuge tube that contained 3-5 ml of foetal bovine serum. After centrifuging the tissue to pellet it, the supernatant was aspirated, and pieces of the pellet were spread out on slides and dried by air. The slides were prepared by being fixed in methanol, dyed in Giemsa, and covered with cover slips that were mounted permanently. All slides were coded before analysis to prevent bias and viewed using an Olympus microscope (Model BX 51) [9].

Assay of micronucleus by using the blood collected through Peripheral route in pregnant rats

Mouse peripheral blood was used to create a small smear on a clean glass slide, which was angled at a 30° angle and lit by a table lamp. The slide was air dried for an hour. The slide was stained with Giemsa after being fixed with 100% methanol for 5 minutes. An Olympus microscope (Model BX 51) was used to view the slides [10].

2. Materials And Methods

Analysis of Slides

The slides were prepared for the examination of micronucleus and calculation of PCE: NCE ratio in treated and control pregnant rats. The bone marrow was collected from six pregnant rats of each group at the scheduled time of treatment. The slides were prepared from those collected bone marrow. From each animal, the minimum number of micro nucleated Polychromatic erythrocytes (PCEs) counted were 2000. These counted PCEs were referred for determining the micronucleus frequency that is expressed as a percentage of micro nucleated cells. The existence of micronuclei was determined using the Schmid criteria. Almond- and ring-shaped micronuclei, which are uncommon, were also found. Typically, micronuclei were round and heavily colored. Micronuclei often possessed crisp edges and a size that varied between twenty-five and one-fifth that of PCEs. The measuring unit was the micro nucleated cell, not the micronucleus, hence sometimes occurring cells with multiple micronuclei were counted as a single micro nucleated PCE rather than as having two or more micronuclei. PCEs and NCEs could be identified by color (bluish-grey and red, respectively) thanks to the staining process [11].

Determination of DNA Damage: Metaphase Chromosome Analysis

The bone marrow cells' mitotic index (MI) was fundamentally established. In a nutshell, pregnant rats were given colchicine (4 mg/kg body weight) treatment approximately 1.5 hours before sacrificing, and then femur bones were separated. Bone marrow was also removed and then treated with a 0.56% KCl solution for 20 minutes at 37°C. The re-suspension was done of the pellet in the solution of Carnoy's fixative after centrifugation (106 g, 7 min). The solution is a combination of methanol and glacial acetic acid in the ratio of 3:1 which means 3 parts of methanol and 1 part of glacial acetic acid. The Pasteur pipette was used for pouring the suspension onto the ice-cold slides. These slides had been stored in a solution of ethanol and water in the ration of 1:1. The slides were then quickly flamed for a short period of time and left to dry at room temperature. Giemsa staining and two phosphate buffer (pH 6.8) washes were performed on the slides [12]. Based on the percentage of dividing cells among the total number of bone marrow cells enumerated, the mitotic index for Cytotoxicity evaluation was derived.

Determination of DNA Damage: DNA Fragmentation Assay

DNA fragmentation was quantified in addition to being analyzed qualitatively by gel electrophoresis by employing the diphenylamine reagent and measuring the absorbance spectrophotometrically. Burton was the first to present this approach. The liver homogenate was mixed with an ice-cold lysis buffer, vortexed, and left to stand at 4°C for 30 minutes. The centrifugation was done of the sample for time period of 15 minutes at the speed of 15,000 revolutions per minute (rpm) at the temperature of 4°C. The solution of Tricarboxylic acid (TCA) was prepared in the concentration of 10% and 5%. The supernatant and pellet were collected separately. From 10% TCA, 1.5 ml were added to the supernatant and from 5%, 0.65 ml was added to the pellet. Both the samples were left for the whole night at 4°C so that precipitation can take place. The heating was given to the pellet solution for 15 minutes after addition of 0.65 ml of centrifuged 5% TCA at 100°C. Each tube further consisted of 1 ml of the diphenylamine reagent after the process of centrifugation. All the tubes were then incubated for 6 hours at 37°C. Finally, a spectrophotometer was used to measure absorbance at 600 nm [13].

Determination of Cytotoxicity: Histological Examination

A transverse piece of the hepatic lobe was obtained from the livers that have been fixed with formalin. The tissue slices of thickness 5 mm were created for the assessment of structure of cell. These slices were stained with haematoxylin and eosin (H & E). A single liver segment from each animal was examined histologically using an Olympus microscope (Model BX 51) [14-31].

Statistical Analysis

Results were shown as Mean ± SEM for each group. The statistical analysis was carried out using Prism Pad and Jandel Sigma Stat (Version 2.03, San Rafael, CA, USA). The significance of the difference between the two groups was evaluated using the student's t-test. To compare numerous variables, one-way analysis of variance (ANOVA) was used. If the ANOVA revealed significant differences, a post hoc analysis using the Dunnett test was carried out. $P<0.05$ was the cutoff for statistical significance.

Haemocrit Analysis

Neumann's chamber was used to measure the total RBC count, leucocyte count, and platelet count in blood samples taken from experimental animals [15].

3. Results and Discussion

Determination of Cytotoxicity: Micronucleus Assay in Peripheral Blood and Bone Marrow

A clear cytotoxic effect of CP on bone marrow proliferation was revealed by measuring the PCE/PCE + NCE ratio in pregnant rats that had been exposed to the substance. Both Table 3 for peripheral blood and Table 1 for bone marrow contain the data. The PCE/PCE + NCE ratio was significantly lower in the PZ treated groups (at doses of 25 mg/kg and 15 mg/kg) than in the control group (p 0.001), whereas it was similar in the PZ treated groups at dose 5 mg/kg.

Table 1: Effect of PZ and CP Treatment on Percentage (%) of MN in Pregnant rats Peripheral Blood

Groups↓	MN %	PCE/(PCE + NCE)%
Control	0.15 ± 0.01	32.58 ± 0.45
Std (CP)	3.22 ± 0.04***	18.80 ± 0.27***
PZ (5mg/kg)	0.21 ± 0.01	30.31 ± 0.39
PZ (15 mg/kg)	1.47 ± 0.02**	26.45 ± 0.35**
PZ (25 mg/kg)	2.57 ± 0.03***	22.76 ± 0.24***

All values are expressed as Mean ± SEM (n = 6/group)

*The statistically significant difference in the level is indicated by **p < 0.01 and ***p < 0.001 by comparison with control group.*

The abbreviations stand for:

MN: Micronucleus, PCE: Polychromatic erythrocyte, NCE: Nor chromatic erythrocyte

Table 2: Effect of PZ and CP Treatment on Percentage (%) of MN in Pregnant rats Bone Marrow

Groups↓	MN %	PCE/(PCE + NCE)%
Control	1.59 ± 0.09	59.08 ± 0.77
Std (CP)	6.48 ± 0.14***	32.53 ± 1.44***
PZ (5 mg/kg)	0.98 ± 0.09	55.45 ± 0.65
PZ (15 mg/kg)	2.77 ± 0.12**	45.39 ± 0.29**
PZ (25 mg/kg)	4.58 ± 0.25***	37.59 ± 0.34***

All values are expressed as Mean ± SEM (n = 6/group)

*The statistically significant difference in the level is indicated by **p < 0.01 and ***p < 0.001 by comparison with control group.*

Effects of PZ on Chromosomal Damage in the Bone Marrow

To evaluate the clastogenic activity of substances, the bone marrow CA test is frequently utilised. Centromeric separations and chromatid gaps were brought on by the PZ treatment in Table 5. Aberrations of various structural and numerical sorts were seen. In comparison to the control group, at larger dosages, such as 1 mg ($P<0.01$), 0.75 mg ($P<0.05$), and Std. (CP) <0.001, PZ treatment significantly increased the number of structural and numerical aberrations. It was discovered that the treated group had an increase in the overall percentage of these abnormalities. This suggests that the DNA is damaged by the medication Prazosin.

Table 3: Effect of PZ and CP Treatment on Chromosomal Aberration Assay on Pregnant rats Bone Marrow

Parameters → Groups↓	Structural aberrations					Gaps	Numerical aberrations		
	Ctb	Csb	Cms	other	Total (%)		pol	end	Total (%)
Control	1	1	1	0	0.75	1	1	1	1
Std (CP 30 mg/kg)	11***	8***	11***	10***	15***	12***	11***	12***	15.5
PZ (5 mg/kg)	1	0	0	0	0	1	1	1	1
PZ (15 mg/kg)	3	2	2	2	5.5**	2	2*	3*	4
PZ (25 mg/kg)	6**	7***	6**	5***	11***	4*	5**	7**	7.5

All values are expressed as Mean ± SEM (n = 6/group)

The statistically significant difference in the level is indicated by $^{**}p < 0.01$ and $^{***}p < 0.001$ by comparison with control group.

The abbreviations stand for:

CTB: chromatid break; **CSB:** chromosome break; **CMS:** centromeric separation; **POL:** polyploidy; **END:** endo-reduplication

Quantitation of Fragmented DNA through the use of Spectrophotometer

In Fig. 1, a considerable rise in the percentage of fragmented DNA was seen in a dose-dependent manner after DNA fragmentation was assessed spectrophotometrically.

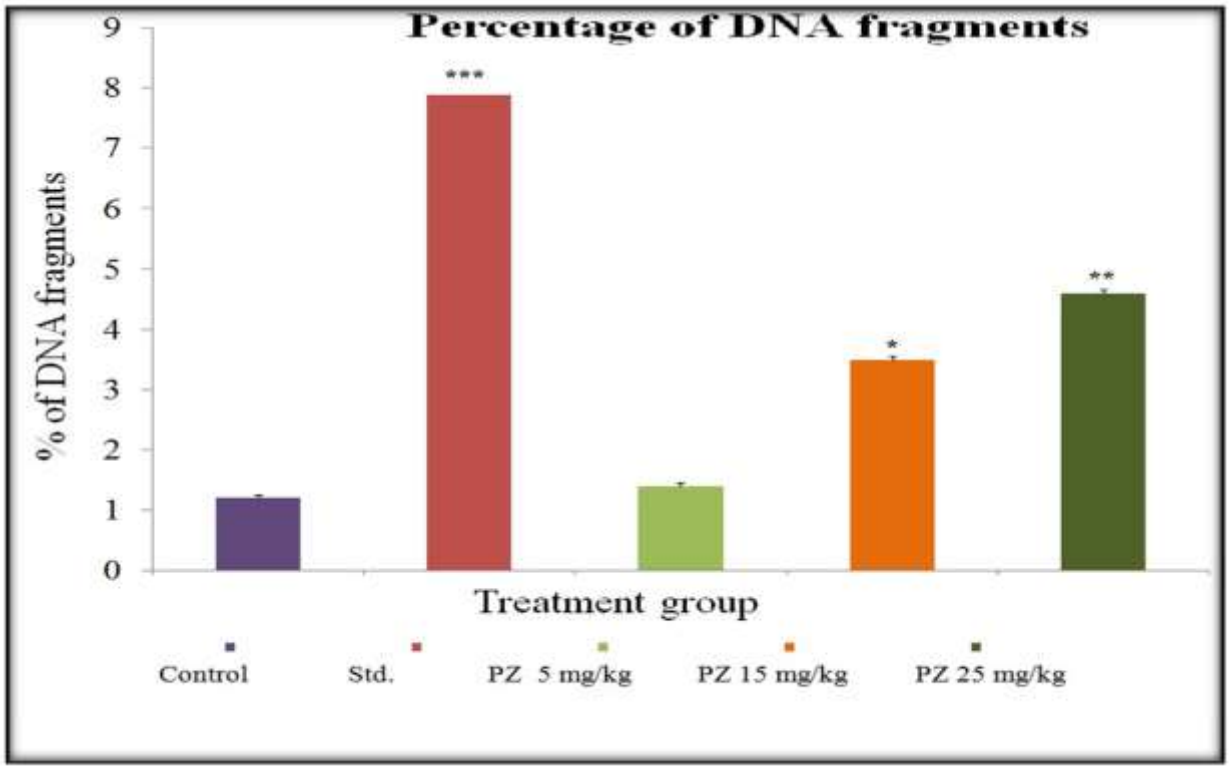

Figure 1: The liver homogenate of different groups showing concentration of DNA fragments in percentage

The statistically significant difference in the level is indicated by $^{**}p < 0.01$ and $^{***}p < 0.001$ by comparison with control group.

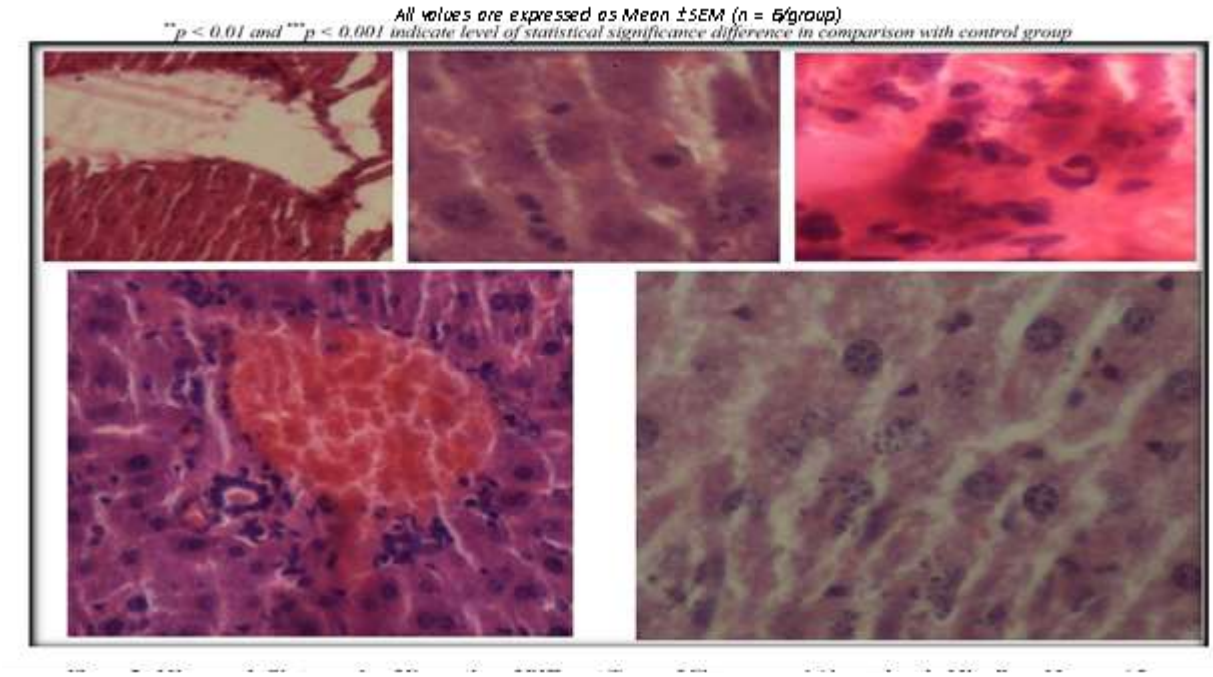

Figure 2: The photographs taken through microscope demonstrating formation of chromosomal aberrations of different types in the Bone Marrow of pregnant rats after they have been treated with Prazosin and Cyclophosphamide.

The portal triad is indicated by the arrows in Section A and C, while the arrows indicate nuclear damage in Section B, D and E

A: Control B: Std. (CP 30 mg/kg) C: PZ (5mg/kg) D: PZ (15 mg/kg) E: PZ (25 mg/kg)

Histological Examination of Liver Sections

The central vein and pinkish portal triad are visible in the control liver section's histological investigation. CP treatment of the liver results in hepatic necrosis, inflammation, clogged venules, degenerative alterations, karyolysis, large and small nuclei, and other degenerative abnormalities. The hepatic venules and pinkish portal triad seen in the control liver segment are also present in PZ-treated animals at the lowest dose (5 mg/kg). PZ treatment (15 mg/kg) results in decreased edema, smudgy cytoplasmic boundaries, no karyolysis, and the presence of inflammatory cells. And the liver segment treated with PZ at the maximum dose (25 mg/kg) demonstrates fluid-filled cells, karyolysis, nuclear and cytoplasmic edema, and localized inflammation; all of these findings are comparable to those seen in the liver section treated with CP. It is evident from Fig. 2 that the medication PZ exhibits toxicity at the greatest dose.

Haemocrit analysis

Results are shown in Table 4.

Table 4: Effect of PZ Treatment on Haemocrit Parameters

Groups↓	RBC (Million/Cmm)	TLC (/Cmm)	PC (lacs/Cmm)
Control	2.28	5,320	0.77
PZ (0.5 mg)	2.40	4,550	0.75
PZ (0.75 mg)	1.17	3,550	0.65
PZ (1mg)	0.42	2,650	0.48

All values are expressed as Mean ± SEM (n = 6/group)

TLC: Total leucocytes count; **PC:** Platelet counts; **RBC:** Red Blood Cell

The antihypertensive drug, Prazosin was reported to possess genotoxic and cytotoxic effects on the bone marrow and liver cells of mouse in the study. The administration of higher doses of the drug was proved to be dangerous to the life of pregnant rats on a systemic level. Because the incidence of structural abnormalities was greater than 10% at two higher dosages of PZ (15 and 25 mg/kg), PZ was judged positive in the in vivo chromosomal aberration test. Additionally, PZ demonstrated positive outcomes in the DNA fragmentation experiment and the mouse bone marrow and peripheral blood micro nucleus (PBMN) test, indicating that PZ destroys DNA in vivo at higher dose concentrations. PCE/(PCE+ NCE)%) decreased in the bone marrow and peripheral blood of the PZ-treated groups.

In the in vivo chromosomal aberration test, PZ was classified as positive because at two higher dosages of PZ (15 and 25 mg/kg), the incidence of structural abnormalities was over 10%. Additionally, PZ tested positive in both the DNA fragmentation assay and the mouse bone marrow and peripheral blood micro nucleus (PBMN) test, indicating that at higher dose concentrations, PZ destroys DNA in vivo. In the PZ-treated groups, there was a decline in PCE/(PCE + NCE)% both the peripheral blood and bone marrow. The bone marrow micro nucleus (BMMN) result was around 22% at the highest dose (25 mg/kg) and 14% at the medium dose (15 mg/kg) in contrast to the control group.

4. Conclusion

In pregnant rats' liver homogenate, the percentage of fragments of DNA increased with increasing test doses of prazosin (0.75 mg/kg and 1 mg/kg), indicating DNA damage. As the liver slice exhibits karyolysis and the development of cytoplasmic edema, histopathological investigation complements the other data to draw the conclusion that at the higher doses PZ exhibited genotoxic and cytotoxic effects. Apoptosis, or "programmed cell death," is crucial for maintaining the steady state in tissues that are constantly regenerating. When compared to the control, PZ significantly increased the amount of DNA fragments at the two higher doses. A post-marketing survey revealed that prazosin may produce a low platelet count (which could lead to bleeding issues).

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