Section A-Research paper
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Research Article

In-Vivo Genotoxicity and Cytotoxicity Study of Prazosin HCl in Pregnant Mice

Sushama Rawat[1], Dipti B Ruikar[2*], Santosh Amritlal Raotole[3], Geeta Sahu[4], Rwitabrata Mallick[5], Manish Kumar Shakya[6], Saroj Kumar Raul[7], S.Varalaxmi[8]

[1]Institute of Pharmacy, Nirma University S G Highway, Ahmedabad & School of Pharmaceutical Sciences, Jaipur National University, Jaipur

[2]P R Pote Patil College of Pharmacy, Amravati, Maharashtra

[3]Sanjivani Institute of Pharmacy and Research, Kopargaon, Shinganapur, Ahmednagar, Maharashtra

[4]St. Wilfred Institute of Pharmacy, Near Shedung, Toll Plaza, Panvel

[5]Department of Environmental Science, Amity School of Life Science, Amity University, Madhya Pradesh, Gwalior

[6]I.T.S College of Pharmacy, Murad Nagar, Ghaziabad, Uttar Pradesh

[7]Maharajah's College of Pharmacy, Phoolbaugh, Vizianagram, Andhra Pradesh

[8]MB School of Pharmaceutical Sciences (Erst While) Sree Vidyanikethan College of Pharmacy, Mohan Babu University, Sree Sainathnagar, Rangampet, Tirupati, Andhra Pradesh

Main Author: Sushama Rawat

sushamar07@gmail.com

Corresponding Author: Dipti B Ruikar*

dipti21ruikar@gmail.com

Abstract:

This research's objective is to examine and assess the cytotoxicity and genotoxicity of prazosin HCL in pregnant mice. 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 investigation was conducted using a variety of techniques, including estimations of the levels of reduced glutathione (GSH), Malondialdehyde (MDA), body weight, organ weight, and food intake. The following parameters have been examined for evaluating genotoxicity: DNA fragmentation assay for determining DNA damage, metaphase chromosomal analysis. The collected information conclusively demonstrates that PZ was harmful to hepatocytes at the higher dose as indicated by elevated MDA levels, decreased GSH levels, DNA damage, and elevated DNA fragmentation in pregnant mice. It's also intriguing to see that PZ caused considerable DNA strand breaks and structural chromosomal abnormalities in bone marrow cell lines. Therefore, it is regarded as being genotoxic to mouse hepatocytes and bone marrow cells. The current investigation demonstrated that Prazosin, at its corresponding hepatotoxic dose level, caused severe genotoxic effects in pregnant mice.

Keywords: Bone marrow, Cytotoxicity, Prazosin, Genotoxicity, Liver, Pregnant mice, Oxidative stress.

Introduction

A higher blood pressure in the arteries is a defining feature of the chronic medical condition known as hypertension (HTN), sometimes referred to as high blood pressure or arterial hypertension. In order to pump blood through the blood vessels, the heart must exert more effort than usual. The aim of antihypertensive drugs is to prevent problems associated with increase in rate of blood pressure like myocardial infarction and stroke [1]. A sympatholytic medication called prazosin (PZ) is used to treat panic disorder, benign prostatic hyperplasia (BPH), excessive blood pressure, and anxiety. Alpha-adrenergic blockers are what it are [2]. Prazosin specifically targets the -1 receptors on vascular smooth muscle. Norepinephrine's vasoconstrictive function, which ordinarily raises blood pressure and intensifies anxiety and panic, is mediated by these receptors. Thus, the drug is able to decrease the rate of blood pressure by blocking the receptors and ultimately reduces anxiety and panic. The drug had been effectively used in the treatment of post-traumatic stress disorder (PTSD) in 10 veterans of the Vietnam War. The study was designed across a 30-week double-blind crossover protocol with a three-week drug interruption to allow for remission [3]. The patients have also been given warning not to rise too quickly. This is because patients might faint due to increased flow of blood to the feet due to weakening in the baroreflex [4]. Vascular enlargement in the nasal mucosa is what causes the nasal congestion.

The "first dose response" is a prazosin-related phenomena where the drug's negative effects, particularly orthostatic hypotension and fainting, are most noticeable

after the first dose. Prazosin (and Prazosin) can extremely rarely cause priapism [5]. Chemotherapy agents called genotoxic medications have an impact on nucleic acids and change how they function [1]. Because they are continually producing new DNA, rapidly dividing cells are especially vulnerable to genotoxic substances.

A cell will frequently go through apoptosis, which is cellular suicide, if enough damage is done to its DNA [6]. During the anaphase of mitosis or meiosis, a micronucleus, an irregular (third) nucleus, is produced. The cytoplasmic formations known as micronuclei, whose name translates to "small nucleus," contain an entire or a portion of an acentric chromosome that was not transported to the opposite poles during anaphase [2]. The daughter cell is left with one or more chromosomes missing as a result of its creation. These chromosome fragments or complete chromosomes typically produce micronuclei, which function as a third nucleus and grow nuclear membranes [7].

Experimental animals

The Institutional Animal Ethics Committee (IAEC) gave its approval to each and every animal experiment. Male Swiss albino mice were purchased from the Central Animal Facility of the Institute for the experiments. The study employed 7-week-old Swiss albino pregnant mice 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 mice in each group for the objective of evaluating cytotoxicity and genotoxicity investigations in Swiss albino adult healthy pregnant mice 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.

Assessment of Food Intake, Organ Weight, and Body Weight

Every day, food intake was recorded, and every other day, body weight was calculated. As the experimental animals ate their meals, care was taken to separate the spilled food from the husk to assess food intake accurately [8]. On the fifteenth day following the slaughter of the animals for each group, the liver weight was calculated.

Preparation of Liver Homogenate

A tube containing 1g of pregnant mouse liver and 4.5 ml of pH 7.4 phosphate buffers was used. After homogenizing the sample with a tissue homogenizer, it was centrifuged for 10 minutes at 7000 g at 4 °C. The supernatant was collected, and Malondialdehyde (MDA), a GSH level, and a lipid peroxidation measurement were all calculated using it [9].

Estimation of Malondialdehyde (MDA) Level

The supernatant of the liver homogenate was removed, and a volume of 2 ml was created by mixing 750 ml of 0.8% thiobarbituric acid (TBA), 100 mL of 8.1% SDS and 750 mL of 20% acetic acid, together. The water bath maintained at temperature 95°C was used for cooking the solution for the duration of 60 minutes [8]. When the test tube was removed and cooled with running water, the sample's color changed to a reddish hue. The sample of solution was again centrifuged for the time period of 10 minutes at 10,000 revolutions per minute (rpm). The absorbance was measured of the sample with the help of instrument spectrophotometer at 532 nm. The analysis of the results was done using a

standard curve. The percentage of the control was used for the presentation of the results [9].

Estimation of Glutathione Reduced (GSH) Level

500 L of chilled, 5% sulfosalicylic acid was then added to the liver homogenate after the supernatant had been removed. It was vortexed before spending 30 minutes on ice. The sample of solution was again centrifuged for the time period of 10 minutes at 10,000 revolutions per minute (rpm) [10]. The supernatant is stored in the freezer separate from the pellet. For the test, 50 ml of the sample was added to 450 ml of pH 7.4 PB. A test tube was filled with 500 ml of PB (pH 7.4) to serve as the blank [8]. Using seven test tubes that were pH 7.4 and contained varying quantities of standard, GSH, and PB. There were vortices in every test tube. Three times, for a total of 500 ml, "Ellman's reagent" was added and vortexed. Reaction time was allocated 10 minutes. The absorbance was measured of the sample with the help of instrument spectrophotometer at 412 nm [11].

The bone marrow cells' mitotic index (MI) was fundamentally established [13, 14]. In a nutshell, pregnant mice were given a colchicines (4 mg/kg body weight) treatment 1.5 hours before being killed, and femur bones were separated. After being extracted, the bone marrow was exposed to a 0.56% KCl solution for 20 minutes at 37° C. The pellet was again suspended in the Carnoy's fixative solution after the process of centrifugation has been completed. The solution of Carnoy's fixative consists of a mixture of 3 portion of methanol and 1 portion of glacial acetic acid. The application of the suspension was done to the ice-cold slides with the help of Pasteur pipette. The flame was introduced to the slides for few seconds and then were cured at room temperature [12].

DNA Fragmentation Assay for the Determination of DNA Damage

The method of gel electrophoresis was used along with the diphenylamine reagent for determining the fragmentation in DNA of pregnant mice. The researcher Burton introduced the process for the first time in the field of cytotoxicity. The homogenate of mice liver was combined with an ice-cold lysis buffer. The combination of sample then vortexed, and left at that position at 4°C for 30 minutes [15]. The supernatant and pellet were separated after centrifuging for approximately 15 minutes at 15,000 rpm at the temperature of 4°C. The 10 % solution of Trichloroacetic acid (TCA) was added to the supernatant in the quantity of 1.5 ml. On the other side, the 5% solution of Trichloroacetic acid (TCA) was added to the pellet in the quantity of 0.65 ml. Both the samples were left for the whole night at the temperature of 4°C to give them the time to fully precipitate. In the next step, the 5% solution of TCA that has been centrifuged was added to the pellet in the quantity of 0.65 ml. Once again the heating was done of the solution for the duration of 15 minutes at the temperature of 100°C. The reagent, diphenylamine was added after centrifuging each of the tubes. The incubation period of 6 hours was provided then to the tubes at the temperature of 37°C. The last step of the process involves measurement of absorbance at 600 nm with the help of spectrophotometer [16-51].

Analytical Statistics

The tabulation of the results was done in the form of Mean ± SEM for each of the groups. The software's like Prism Pad and Jandel Sigma Stat (Version 2.03, San Rafael, CA, USA) was referred for carrying out the statistical analysis. The Students t-test software was used for evaluating the significance of the difference between the two groups. To compare numerous variables, one-way analysis of variance (ANOVA) was used.

Results

Body Weight, Food Intake, and Liver Weight

From the results, it can be concluded that the difference in the body weight of animals at the doses of 5, 15 and 25 mg/kg was statistically significant 14 days of Prazosin (PZ) treatment when compared to the control ($P<0.05$). When compared to the corresponding control in Table 2, PZ therapy at a dose of 5 mg/kg resulted in considerably less overall food consumption ($P<0.001$). When compared to the corresponding control in Fig. 1, the effects of DZ therapy at the dose of 5 mg/kg on liver weight were substantial ($P<0.001$).

Table1: Effect of PZ and CP Treatment on Pregnant mice Body Weight

Treatment→Days↓	Control	Std (CP)	PZ(5mg/kg)	PZ(15mg/kg)	PZ(25mg/kg)
1st	40.3±0.04	40.5±0.52	40.6±0.09	40.5±0.17	40.5±0.34
3rd	40.4±0.04	40.5±0.52	40.6±0.09	41.0±0.16	40.3±0.34
5th	40.4±0.03	40.4±0.52	40.4±0.07	40.8±0.14	40.4±0.34

7th	40.4±0.03	40.6±0.53	40.3±0.10	31.8±0.17	32.5±0.36
9th	40.2±0.03	40.5±0.52	40.2±0.09	29.4±0.15	31.2±0.33
11th	40.2±0.04	40.5±0.53	40.1±0.08	28.3±0.15	28.7±0.34
14th	40.3±0.04	38.5±0.55***	31.4±0.05	31.0±0.30*	30.1±0.30**

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

The level of statistical significance difference is indicated by comparing with control group by **p<0.01and ***p<0.001.

PZ: Prazosin, **CP:** Cyclophosphamide

Table2: Effect of PZ and CP Treatment on Pregnant mice Food Intake

Days↓	Control	Std(CP)	PZ(0.5mg)	PZ(0.75mg)	PZ(1mg)
1st	17.2±0.03	17.4±0.02	17.3±0.03	17.4±0.02	17.3± 0.04
2nd	17.3±0.02	17.1±0.02	17.2±0.04	17.2±0.04	17.3± 0.04
3rd	17.4±0.03	17.3±0.08	17.3±0.13	17.1±0.06	17.2±0.13
4th	17.4±0.04	17.2±0.03	17.3±0.02	17.4±0.11	17.6± 0.12
5th	17.2±0.05	17.3±0.02	17.9±0.08	17.5±0.05	17.4±0.15
6th	17.3±0.03	17.2±0.01	17.8±0.12	17.6±0.05	17.1±0.15
7th	17.4±0.14	17.8±0.10	17.7±0.02	17.5±0.03	17.2± 0.14
8th	17.3±0.10	17.9±0.15	16.9±0.11	17.3±0.15	15.7± 0.18
9th	17.3±0.20	17.9±0.15	17.6±0.11	17.2±0.04	15.9± 0.07
10th	17.1±0.02	16.9±0.09	17.4±0.07	17.0±0.07	15.8± 0.06
11th	17.2±0.13	16.7±0.11	17.9±0.07	16.9±0.03	15.5±0.20
12th	17.9±0.12	15.9±0.13	17.8±0.09	15.9±0.10	1.5.2±0.17
13th	17.8±0.04	14.9±0.06	17.1±0.10	15.5±0.15	15.9± 0.23
14th	17.6±0.06	15.1±0.06***	17.4±0.12	15.2±0.15**	16.4±0.24***

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

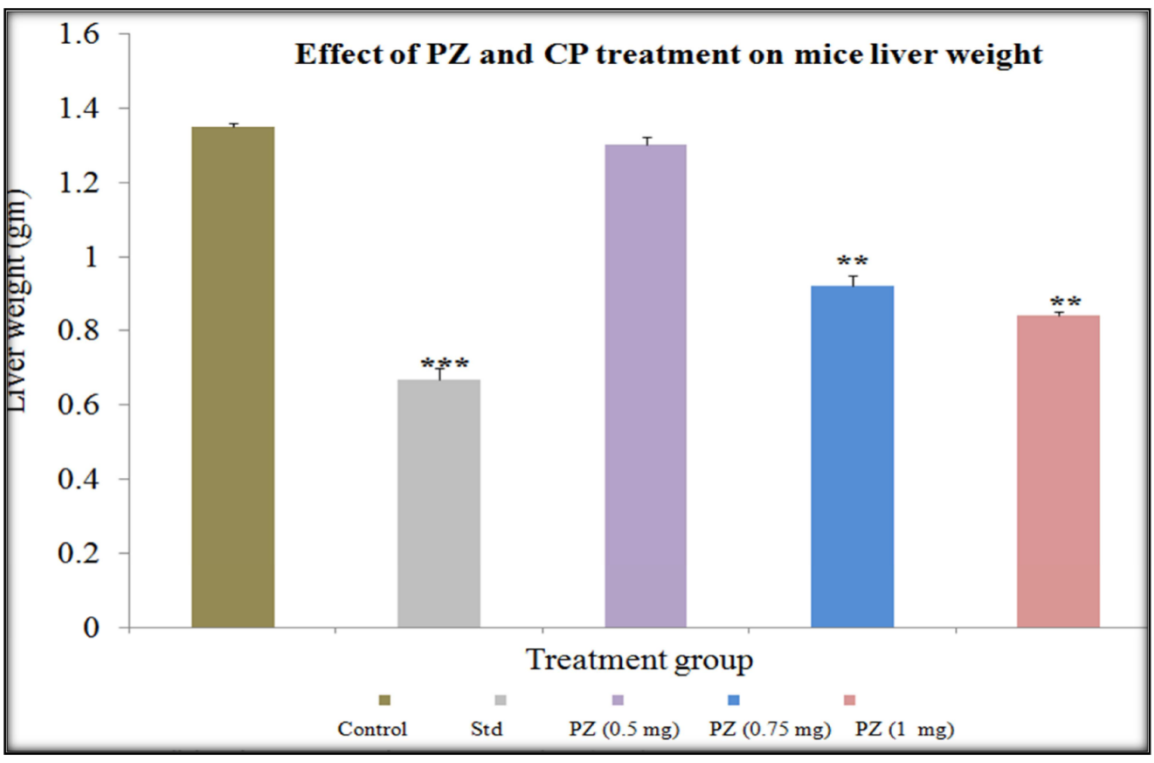

Figure1: Effect of PZ and CP treatment on pregnant mice Liver Weight

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

The level of statistical significance difference is indicated by comparing with control group by **p<0.01and ***p<0.001.

Estimation of MDA and GSH Level in Liver Homogenate

Treatment with prazosin resulted in a considerable rise in MDA levels and a fall in GSH levels. In Figures 2 and 3, P < 0.001 at the dose of 25 mg/kg and P < 0.05 at the dose of 15 mg/kg were both calculated in comparison to the control.

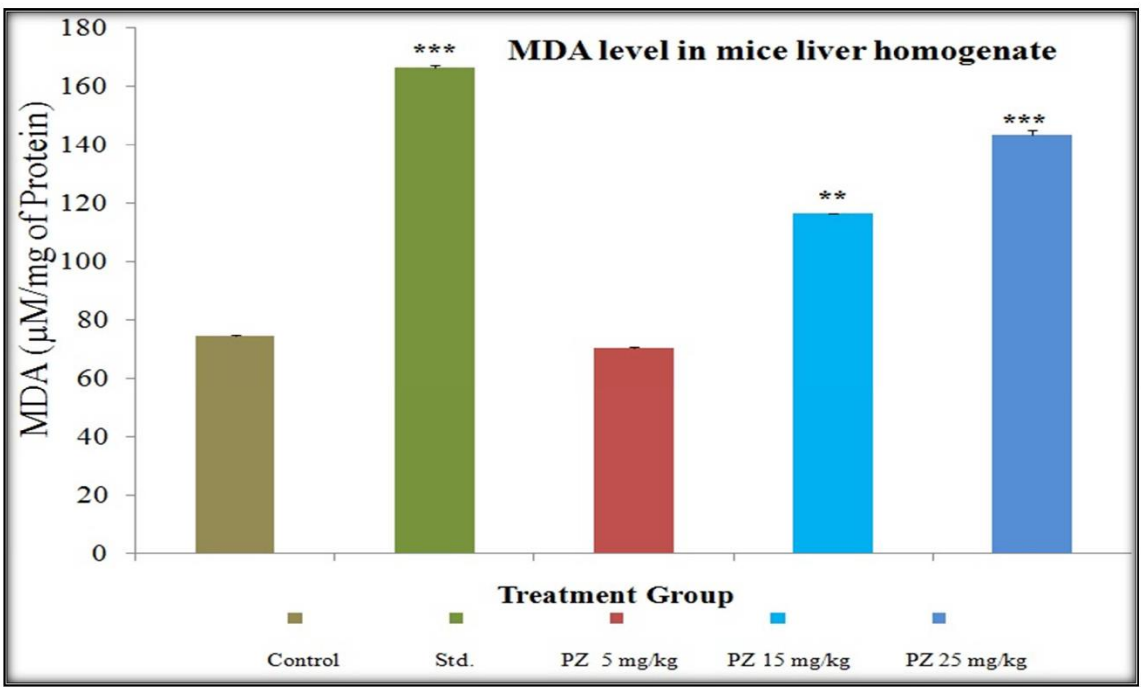

Figure2: MDA Level in Liver Homogenate in the Treatment Groups

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

The level of statistical significance difference is indicated by comparing with control group by $^{**}p<0.01$and $^{***}p<0.001$.

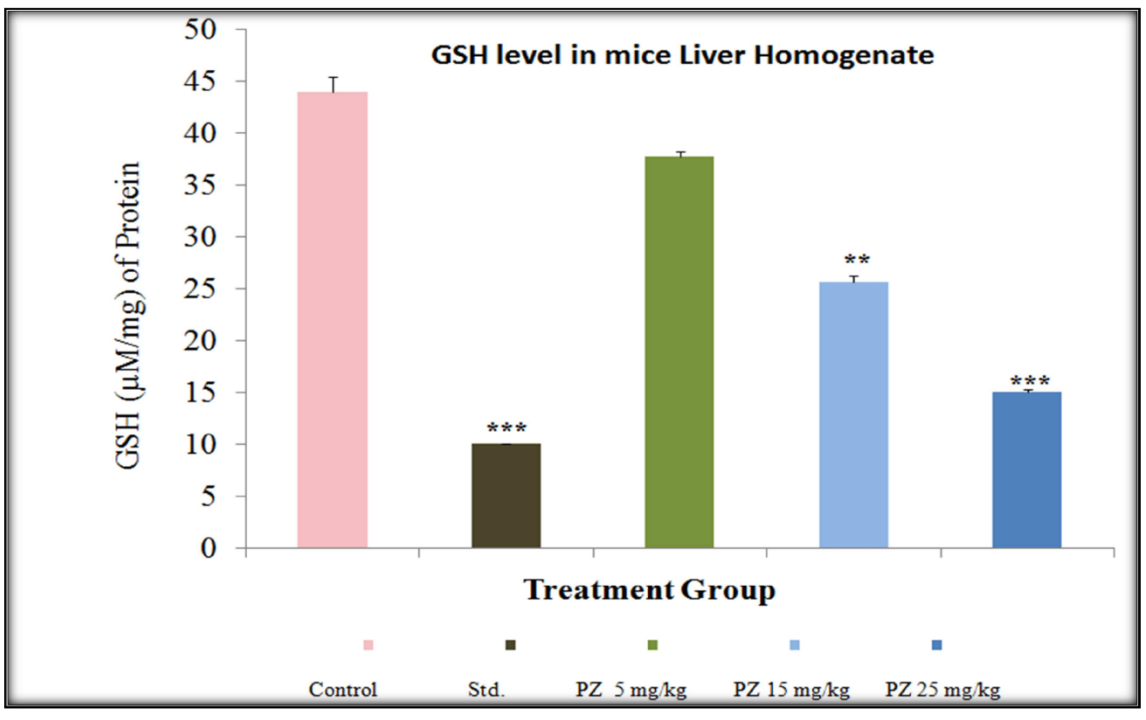

Figure3: GSH Level in Liver Homogenate in the Treated Groups

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

The level of statistical significance difference is indicated by comparing with control group by $^{**}p<0.01$and $^{***}p<0.001$.

Effects of PZ on Chromosomal Damage in the Bone Marrow

To evaluate the clastogenic activity of substances, the bone marrow CA test is frequently utilized. Centromeric separations and chromatid gaps were brought on by the PZ treatment in Table 3. Aberrations of various structural and numerical sorts were seen. In compared to the control group, PZ administration significantly increased the number of structural and numerical aberrations at higher dosages, such as 1 mg ($P < 0.01$) and 0.75 mg ($P < 0.05$) and Std. (CP) < 0.001. 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.

Table3: Effect of PZ and CP Treatment on Chromosomal Aberration Assay on Pregnant mice Bone Marrow

Parameters→ Groups↓	**Structural aberrations**					**Gaps**	**Numerica laberrations**		
	Ctb	**Csb**	**Cms**	**other**	**Total (%)**		**pol**	**end**	**Total (%)**
Control	0.1	0.2	1.2	0.1	0.25	1	0.1	0.1	0.25
Std(CP30mg/kg)	11***	8***	10***	9***	15***	9***	8***	11***	14.5
PZ(5 mg/kg)	0.1	1.2	0.2	1.3	1.4	0.5	1.1	0.2	1.2
PZ(15mg/kg)	4.2	3.3	3.2	3.1	6.4**	3.1	2.7*	3.8*	4.9
PZ(25mg/kg)	7.2**	5.6***	6.2**	6.4***	11.8***	5.6*	5.7**	8.2**	8.3

AllvaluesareexpressedasMean±SEM(n=6/group)

The level of statistical significance difference is indicated by comparing with control group by $^{**}p<0.01$and $^{***}p<0.001$.

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

Quantitation of Fragmented DNA by Spectrophotometry

In Fig. 4, a considerable rise in the percentage of fragmented DNA was seen in a dose-dependent manner after DNA fragmentation was assessed spectrophotometrically.

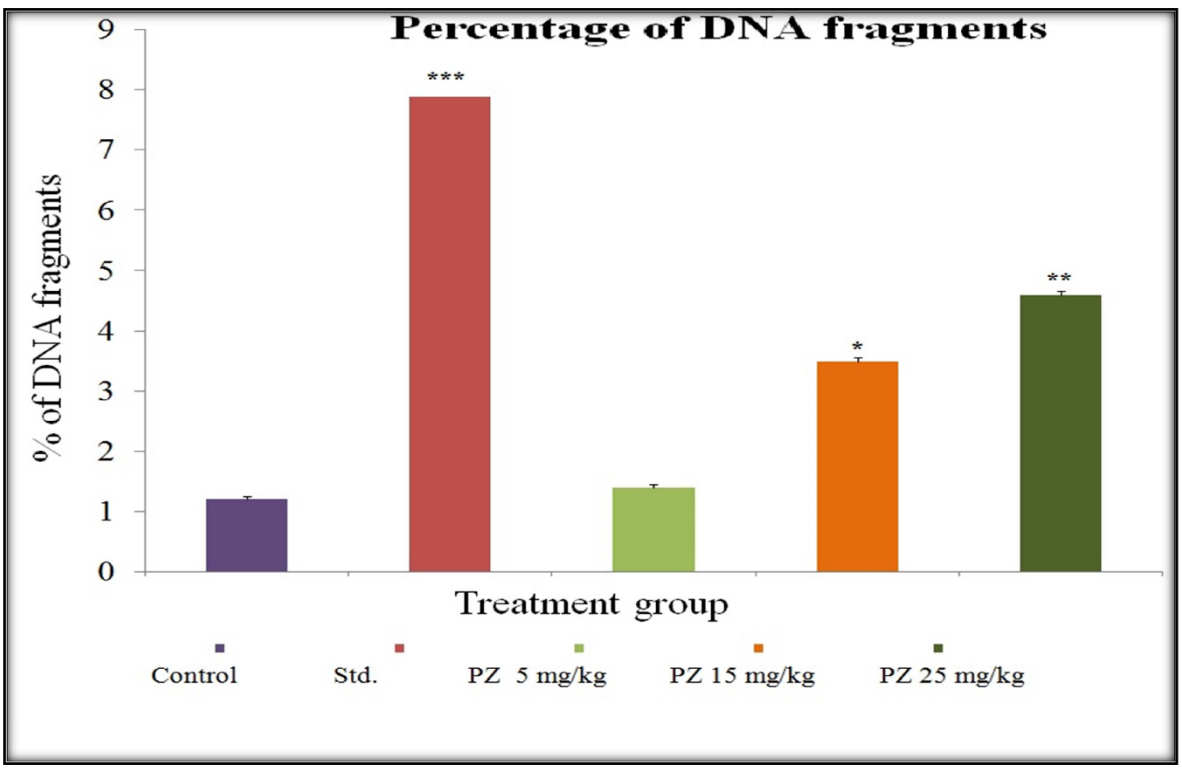

Fig 4: Percentage of DNA fragments in Liver Homogenate

Discussion

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 mice on a systemic level. This was evident from the marked decrease in the body weight, intake of food and weight of liver to the statistical significant level among the treatment groups. The result was compared to the control groups. The drug, Prazosin is metabolized mainly through liver. The bioavailability of Prazosin is reported to be 56.9% with the variation of range from 43.5% to 69.3% when administered through oral route. Prazosin is highly (92–97%) bound to albumin and alpha 1-acid glycoprotein in human plasma, and the level of binding is unrelated to the drug's plasma concentration, which ranges from 20–150 ng/ml. PZ was deemed positive in the in vivo chromosomal aberration test because, at two higher doses of PZ (15 and 25 mg/kg), the incidence of structural abnormalities was more than 10%. Additionally, PZ showed positive results in the mouse bone marrow and peripheral blood micro nucleus (PBMN) test as well as the DNA fragmentation assay, demonstrating that PZ damages DNA in vivo at higher dose concentrations. Both the bone marrow and peripheral blood of the PZ-treated groups showed a drop in PCE/(PCE+ NCE)%).

In pregnant mice liver homogenate, the percentage of DNA fragments 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 PZ is genotoxic and cytotoxic at two higher doses. 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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