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In-Vivo Genotoxicity and Cytotoxicity Study of Prazosin HCl

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ABSTRACT

The aim of performing this work is to study and evaluate Genotoxicity and Cytotoxicity of Prazosin HCL in mice. The animals were treated with Prazosin (PZ) at the doses of 5, 15, 25 mg/kg/body weight intraperitoneal (IP) for single dose (14 days) toxicity studies. Different methods were used to perform the study like Measurement of body weight, organ weight and food intake, Estimation of Malondialdehyde (MDA) level, Estimation of Reduced Glutathione (GSH) level, For evaluation of Genotoxicity following parameters has been evaluated: Micronucleus assay in bone marrow, Micronucleus assay in peripheral blood, Determination of DNA damage: Metaphase chromosome analysis, Determination of DNA damage: DNA fragmentation assay, Determination of Cytotoxicity: Histological examination.The results obtained clearly demonstrate that PZ produced toxic responses at the higher dose in the hepatocytes as evident from increased MDA level, decreased GSH level, DNA damage, increased DNA fragmentation in mice. Also, it is interesting that in bone marrow cells, PZ induced structural chromosomal aberrations, and significantly DNA strand breakage observed. So it is considered as Genotoxicity toward the bone marrow cells and to the hepatocytes of mice. The present study provided evidence that Prazosin induced significant genotoxic effects in mice at its equivalent hepatotoxic dose level.

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

INTRODUCTION

Hypertension (HTN) or high blood pressure, sometimes called arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated. This requires the heart to work harder than normal to circulate blood through the blood vessels. Antihypertensives are a class of drugs that are used to treat hypertension (high blood pressure). Antihypertensive therapy seeks to prevent the complications of high blood pressure, such as stroke and myocardial infarction [1]. Prazosin (PZ) is a sympatholytic drug used to treat high blood pressure and anxiety, Benign Prostatic Hyperplasia (BPH) and panic disorder. It is an alpha-adrenergic blocker [2]. Specifically, prazosin is selective for the α-1 receptors on vascular smooth muscle. These receptors are responsible for the vasoconstrictive action of norepinephrine, which would normally raise blood pressure and cause increase in anxiety and panic. By blocking these receptors, prazosin reduces blood pressure and reduces anxiety and panic. The efficacy of prazosin for PTSD among ten Vietnam combat veterans in a 20-week double-blind crossover protocol with a two-week drug washout to allow for return to baseline [3]. It is also reported that patients on prazosin should be told not to stand up too quickly, since their poor baroreflex may cause them to faint as all their blood rushes to their feet [4]. The nasal congestion is due to dilation of vessels in the nasal mucosa. One phenomenon associated with prazosin is known as the "first dose response", in which the side effects of the drug, especially orthostatic hypotension and fainting, are

especially pronounced in the first dose. Priapism as a very rare side effect of Prazosin (and doxazosin) [5]. Genotoxic drugs are chemotherapy agents that affect nucleic acids and alter their function. These drugs may directly bind to DNA or they may indirectly lead to DNA damage by affecting enzymes involved in DNA replication. Rapidly dividing cells are particularly sensitive to genotoxic agents because they are actively synthesizing new DNA. If enough damage is done to the DNA of a cell it will often undergo apoptosis, the equivalent of cellular suicide [6]. Micronucleus is an erratic (third) nucleus that is formed during the anaphase of mitosis or meiosis. Micronuclei (the name means 'small nucleus') are cytoplasmic bodies having a portion of acentric chromosome or whole chromosome which was not carried to the opposite poles during the anaphase. Their formation results in the daughter cell lacking a part or all of a chromosome. These chromosome fragments or whole chromosomes normally develop nuclear membranes and form as micronuclei as a third nucleus [7].

EXPERIMENTAL SECTION

Experimental Animals

All the animal experiments were approved by the Institutional Animal Ethics Committee (IAEC). Experiments were performed on male Swiss albino mice that were procured from the Institute's Central Animal Facility. Swiss albino mice (7 weeks, weighing around 25–30 g) were used in this study. These animals were kept under controlled environmental conditions at room temperature (22 ± 2°C), humidity (50 ± 10%), and automatically controlled 12 hr light and 12 hr dark cycles. Standard laboratory animal feeds were purchased from a commercial supplier and water was given to the animals and libitum. Animals were acclimatized to the experimental conditions prior to the start of dosing for a period of 2-3 days**.**

Chemicals

All the chemicals and reagents used to carry out the research work were of analytical grade.

Drugs

Prazosin purchased from Sigma Aldrich, Cyclophosphamide was purchased from Hi-Media. 5% Sulfosalicylic acid, ThioBarbituric Acid (TBA), Ellman's solution (5, 5-dithiobis-2-nitrobenzoic acid), Colchicine, Diphenylamine, Foetal Bovine Serum (FBS), Bovine Serum Albumin (BSA). All these chemicals were purchased from Sigma Aldrich.

Rationality of Dose Selection

In this investigation, Prazosin (PZ) was administered at the doses of 5, 15, 25 mg/kg. The dose was carefully selected based on the earlier studies conducted in animals as well as based on the human equivalent dose.

Treatment Protocol

For evaluation of Genotoxicity and Cytotoxicity studies of Prazosin in Swiss albino adult healthy mice, animals were divided into 5 groups (n=6/group), in the following manner:

Group 1 (Normal control): received de ionized water (2 ml/kg; i.p.) once daily for 14 days.
Group 2 (Standard): received Cyclophosphamide (30 mg/kg; i.p.) dissolved in distilled water once on the 12^{th} day of the 14 days study.
Group 3 (PZ): received Prazosin (5 mg/kg; i.p.) dissolved in de-ionized water on slight heating once daily for 14 days.
Group 4 (PZ): received Prazosin (15 mg/kg; i.p.) dissolved in de-ionized water on slight heating once daily for 14 days.
Group 5 (PZ): received Prazosin (25 mg/kg; i.p.) dissolved in de-ionized water on slight heating once daily for 14 days.

Measurement of Body Weight, Organ Weight and Food Intake

Body weight was measured on alternate day while food intake was measured on each day. To take an accurate food intake measurement, attention was being given to separate the spillage food from the husk during the course of food consumption by the experimental animals [8]. The liver weight was measured on the 15^{th} day after sacrificing the animals of each group.

Preparation of Liver Homogenate

1g of mice liver was taken in a tube in 4.5 ml of phosphate buffer (pH 7.4). Using tissue homogenizer homogenized it and then centrifuged at 7000 G for 10 min at 4°C.Supernatant was collected and used for the estimation of Malondialdehyde (MDA) as a marker of lipid peroxidation and GSH level.

Estimation of Malondialdehyde (MDA) Level

100 µl of supernatant was taken from liver homogenate and added 100 µl of 8.1% SDS + 750 µl of 20% acetic acid +750 µl of 0.8% thiobarbituric acid (TBA) in a glass tube and made volume up to 2 ml. Heated it over water bath at 95ºC for 60 minutes**.** Then test tube was taken out, cooled under tap water and colour of the sample became pinkish. Again centrifuged at 10,000 rpm for 10 min. Absorbance was taken at 532 nm using spectrophotometer and results were calculated using standard curve and expressed as percentage of control [9].

Estimation of Reduced Glutathione (GSH) Level

500 µl of supernatant was taken from liver homogenate and added 500 µl of 5% chilled sulfosalicylic acid. Vortexed it and kept it in ice for 30 min. Again centrifuged at 10,000 rpm for 10 min. Supernatant separated from pellet and stored in freezer. For test, took 450 µl of PB (pH 7.4) and added 50 µl of sample. For blank, took 500 µl of PB (pH 7.4) in a test tube. For standard, took seven test tubes containing different concentration of standard, GSH and PB (pH 7.4). Vortexed all the test tubes. Added 3 times 500 µl of 'Ellman's reagent' and vortexed. Kept it for 10 min (reaction time). Took absorbance at 412 nm [10].

Evaluation of Genotoxicity

Micronucleus Assay in Bone Marrow

The animals were euthanized by diethyl ether inhalation followed by incision of the diaphragm. The femur was removed for bone marrow extraction from six surviving animals in each treatment and control group. For each animal, the marrow flushed from the bones was combined in an individual centrifuge tube containing 3–5 ml foetal bovine serum (one tube per animal). Following centrifugation to pellet the tissue, the supernatant was removed by aspiration and portions of the pellet were spread on slides and air-dried. The slides were fixed in methanol, and then stained in Giemsa, and protected by permanently mounted cover slips. For control of bias, all slides were coded prior to analysis. Observed using an Olympus microscope (Model BX 51) microscope [11].

Micronucleus Assay in Peripheral Blood

The thin smear on a clean glass slide was prepared using peripheral blood of mice at 30^{o} angle in presence of table lamp. Air dried the slide for 1 hr. The slide was fixed with 100% methanol for 5 min and then stained with Giemsa. The slides were observed using an Olympus microscope (Model BX 51) microscope [11].

Slide Analysis

Slides prepared from the bone marrow collected from five animals per group at the designated harvest time points were scored for micronuclei and the PCE: NCE cell ratio. The micronucleus frequency (expressed as percent micro nucleated cells) was determined by analyzing the number of micro nucleated PCEs from at least 2000 PCEs per animal. The PCE : NCE ratio was determined by scoring the number of PCEs and NCEs observed in at least the first 500 erythrocytes per animal. The historical background frequency of micro nucleated cells was expressed as percent micro nucleated cells based on the number of PCEs analyzed. The criteria for the identification of micronuclei were those of Schmid. Micronuclei were darkly stained and generally round, although almond- and ring-shaped micronuclei occasionally occurred. Micronuclei were sharp bordered and generally between one-twentieth and one-fifth the size of the PCEs. The unit of scoring was the micro nucleated cell, not the micronucleus; thus, occasional cell with more than one micronucleus was counted as one micro nucleated PCE, not two (or more) micronuclei. The staining procedure permitted the differentiation by colour of PCEs and NCEs (bluish-grey and red, respectively) [12].

Determination of DNA Damage: Metaphase Chromosome Analysis

Mitotic index (MI) in the bone marrow cells was essentially determined [13, 14]. Briefly, mice were treated with colchicines (4 mg/kg bw) 1.5 hr prior to killing and femur bones were isolated. Bone marrow was flushed out and incubated at 37° C with 0.56% KCl solution for 20 min. After centrifugation (106 g, 7 min), the supernatant was discarded and the pellet was re-suspended in carnoy's fixative (3:1 mixture of methanol and glacial acetic acid). The suspension was dropped on the ice-cold slides (previously kept in the 1:1 mixture of ethanol and water) using Pasteur pipette, and slides were immediately flamed for few seconds and allowed to dry at the room temperature.

Slides were stained with Giemsa and washed twice with phosphate buffer (pH 6.8) [15]. The mitotic index for Cytotoxicity evaluation was calculated on the basis of percentage of dividing cells out of the total bone marrow cells counted.

Determination of DNA Damage: DNA Fragmentation Assay

In addition to qualitative analysis by gel electrophoresis, DNA fragmentation was quantitatively determined by using the diphenylamine reagent and measuring the absorbance spectro photo metrically. This method was first introduced by Burton. An ice-cold lysis buffer was added to the liver homogenate, vortexed, and allowed to stand for 30 min at 4°C. After centrifugation at 15,000 rpm for 15 min at 4°C, 1.5 ml of 10% TCA was added to the supernatant and 0.65 ml of 5% TCA to the pellet. Both samples were allowed to precipitate for overnight at 4°C. Again, centrifuged 0.65 ml of 5% TCA was added to the pellet and boil for 15 min at 100°C. After centrifugation, 1 ml of diphenylamine reagent was added to each tube and incubated at 37°C for 6 hr. Finally, absorbance was taken at 600 nm using spectrophotometer [16].

Determination of Cytotoxicity: Histological Examination

A transverse section of the hepatic lobe was collected from formalin fixed livers. Paraffin-embedded tissue sections were prepared at a thickness of 5 µm and stained with haematoxylin and eosin (H & E) for the evaluation of cellular structure. All the histological examinations were performed by evaluating one liver section per animal, using an Olympus microscope (Model BX 51).

Statistical Analysis

Results were shown as Mean ± SEM for each group. Statistical analysis was performed using Jandel Sigma Stat (Version 2.03, San Rafael, CA, USA) and Prism Pad statistical software. Significance of difference between two groups was evaluated using Student's t-test. For multiple comparisons, one-way analysis of variance (ANOVA) was used. In case ANOVA showed significant differences, post hoc analysis was performed with Dunnett test. $P < 0.05$ was considered to be statistically significant.

Haemocrit Analysis

Blood sample was collected from experimental animals and total RBC count, leucocyte count and platelet count were observed using neumann's chamber.

RESULTS

Body Weight, Food Intake and Liver Weight

Significant difference in the final body weight ($P < 0.05$) was observed after 14 days of Prazosin (PZ) treatment at the dose of 5, 15, 25 mg/kg 80 mg/kg when compared to control in Table 1. Total food intake was significantly reduced ($P < 0.001$) as a result of PZ treatment at the dose of 25 mg/kg when compared to respective control in Table 2. Liver weight was significantly reduced ($P < 0.001$) as a result of PZ treatment at the dose of 25 mg/kg when compared to respective control in Fig 1.

Table 1 : Effect of PZ and CP Treatment on Mice Body Weight

Treatment→ Days↓	Control	Std (CP)	PZ (5mg/kg)	PZ (15 mg/kg)	PZ (25 mg/kg)
1st	31.5 ± 0.06	31.5 ± 0.62	31.5 ± 0.09	31.5 ± 0.19	31.5 ± 0.37
3rd	31.6 ± 0.07	31.5 ± 0.62	31.5 ± 0.10	31.2 ± 0.18	31.2 ± 0.36
5th	31.4 ± 0.03	31.6 ± 0.62	31.3 ± 0.08	31.0 ± 0.17	31.1 ± 0.37
7th	31.7 ± 0.02	31.6 ± 0.63	31.2 ± 0.09	30.8 ± 0.19	30.7 ± 0.38
9th	31.3 ± 0.04	31.5 ± 0.62	31.2 ± 0.09	30.5 ± 0.18	30.1 ± 0.39
11th	31.7 ± 0.05	31.5 ± 0.63	31.0 ± 0.07	30.2 ± 0.19	29.7 ± 0.38
14th	31.4 ± 0.03	27.5 ± 0.65***	30.8 ± 0.06	30.0 ± 0.20*	29.0 ± 0.39**

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

**p < 0.05, **p < 0.01 and ***p < 0.001 indicate level of statistical significance difference in comparison with control group.*

***PZ:** Prazosin, **CP:** Cyclophosphamide*

Table 2 : Effect of PZ and CP Treatment on Mice Food Intake

Days↓	Control	Std (CP)	PZ (0.5 mg)	PZ (0.75 mg)	PZ (1 mg)
1st	18.3 ± 0.04	18.4 ± 0.03	18.4 ± 0.04	18.5 ± 0.03	18.5 ± 0.03
2nd	18.4 ± 0.03	18.2 ± 0.03	18.3 ± 0.005	18.3 ± 0.05	18.4 ± 0.004
3rd	18.5 ± 0.04	18.4 ± 0.09	18.3 ± 0.14	18.2 ± 0.08	18.0 ± 0.124
4th	18.5 ± 0.05	18.3 ± 0.04	18.4 ± 0.04	17.8 ± 0.12	17.7 ± 0.11
5th	18.3 ± 0.06	18.4 ± 0.03	18.2 ± 0.09	17.8 ± 0.06	17.5 ± 0.17
6th	18.4 ± 0.02	18.3 ± 0.02	18.1 ± 0.13	17.7 ± 0.07	17.2 ± 0.19
7th	18.5 ± 0.15	18.0 ± 0.12	18.1 ± 0.03	17.6 ± 0.04	17.0 ± 0.13
8th	18.4 ± 0.11	18.2 ± 0.17	17.7 ± 0.12	17.4 ± 0.16	16.9 ± 0.17
9th	18.2 ± 0.19	18.4 ± 0.17	17.6 ± 0.11	17.3 ± 0.06	16.8 ± 0.08
10th	18.2 ± 0.03	17.0 ± 0.10	18.5 ± 0.08	17.1 ± 0.08	16.7 ± 0.05
11th	18.3 ± 0.14	17.8 ± 0.12	18.2 ± 0.08	17.0 ± 0.04	16.4 ± 0.19
12th	18.1 ± 0.13	16.5 ± 0.14	18.0 ± 0.10	16.7 ± 0.13	16.2 ± 0.18
13th	18.8 ± 0.06	15.3 ± 0.05	18.3 ± 0.12	16.6 ± 0.17	15.9 ± 0.23
14th	18.7 ± 0.08	14.9 ± 0.07***	18.4 ± 0.13	16.3 ± 0.16**	15.4 ± 0.24***

All values are expressed as Mean ±SEM (n = 6/group)
***p < 0.01 and ***p < 0.001 indicate level of statistical significance difference in comparison with control group.*

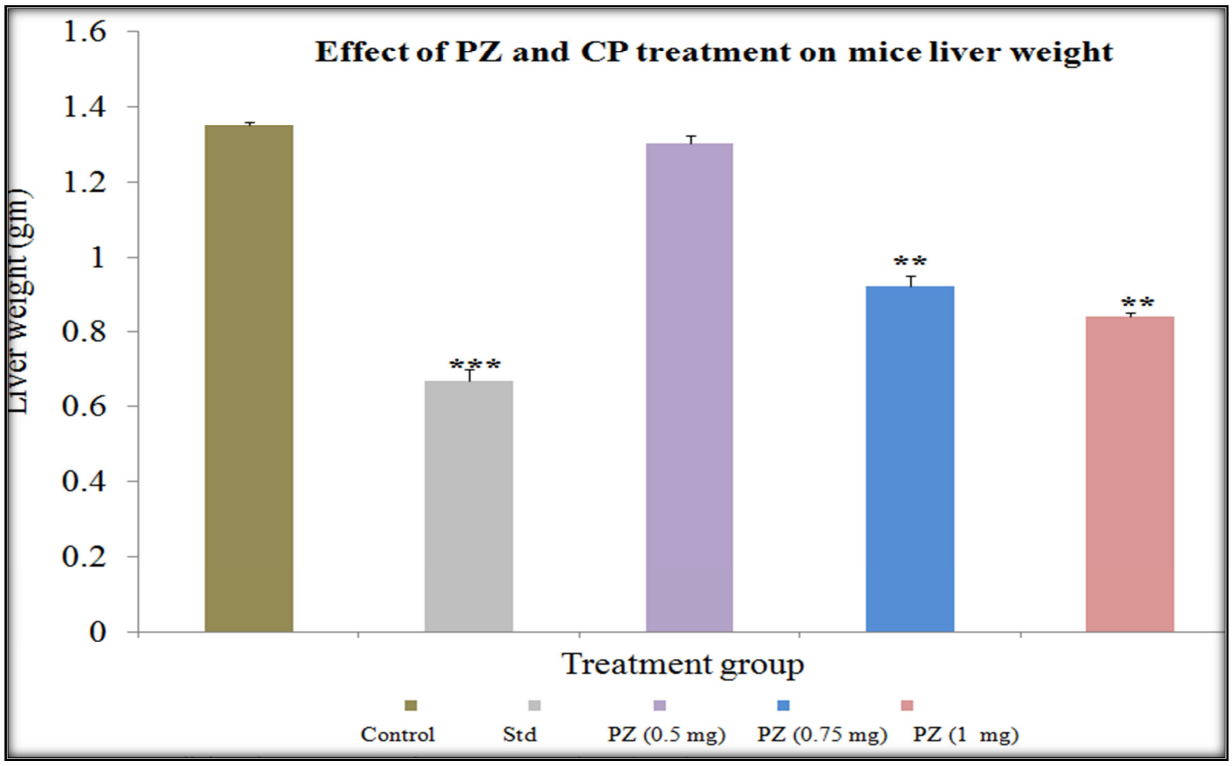

Figure 1 : Effect of PZ and CP Treatment on Mice Liver Weight
All values are expressed as Mean ±SEM (n=6/group)
**p < 0.05, **p < 0.01 and ***p < 0.001 indicate level of statistical significance difference in comparison with control group.*

Estimation of MDA and GSH Level in Liver Homogenate

PZ treatment led to significant increase in the MDA level and decrease in the GSH level. ($P < 0.001$) at the dose of 25 mg/kg and ($p < 0.05$) at the dose of 15 mg/kg when compared to control in Figure 2 and 3.

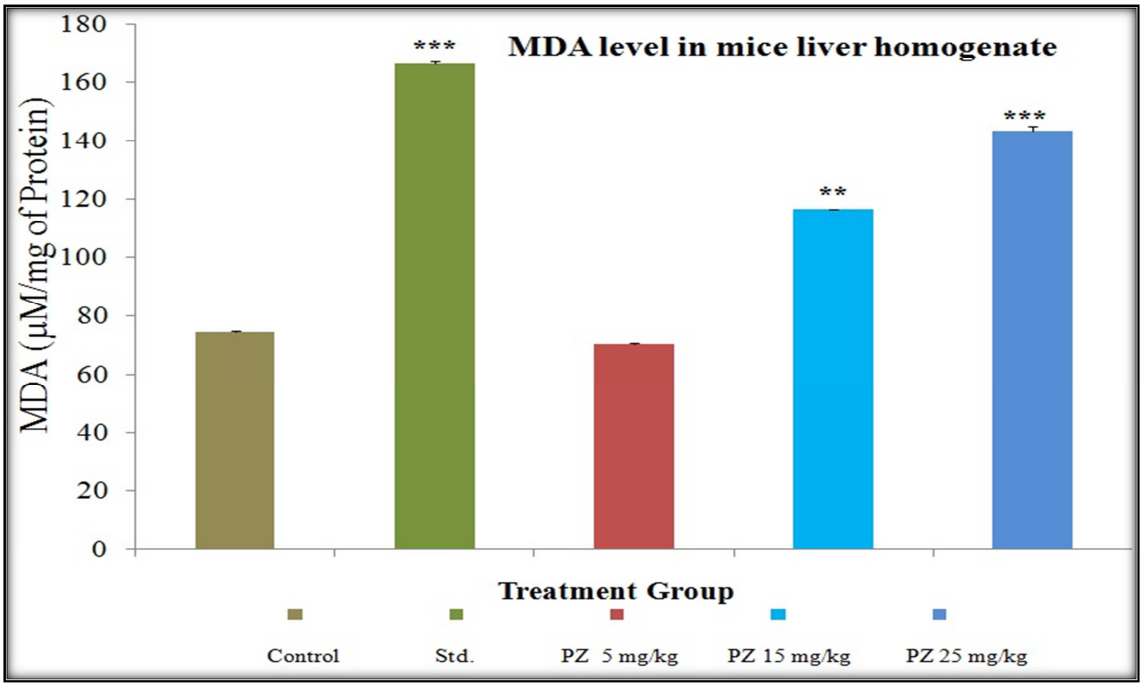

Figure 2 : MDA Level in Liver Homogenate in the Treatment Groups
All values are expressed as Mean ±SEM (n=6/group)
$^{**}p < 0.01$ and $^{***}p < 0.001$ indicate level of statistical significance difference in comparison with control group

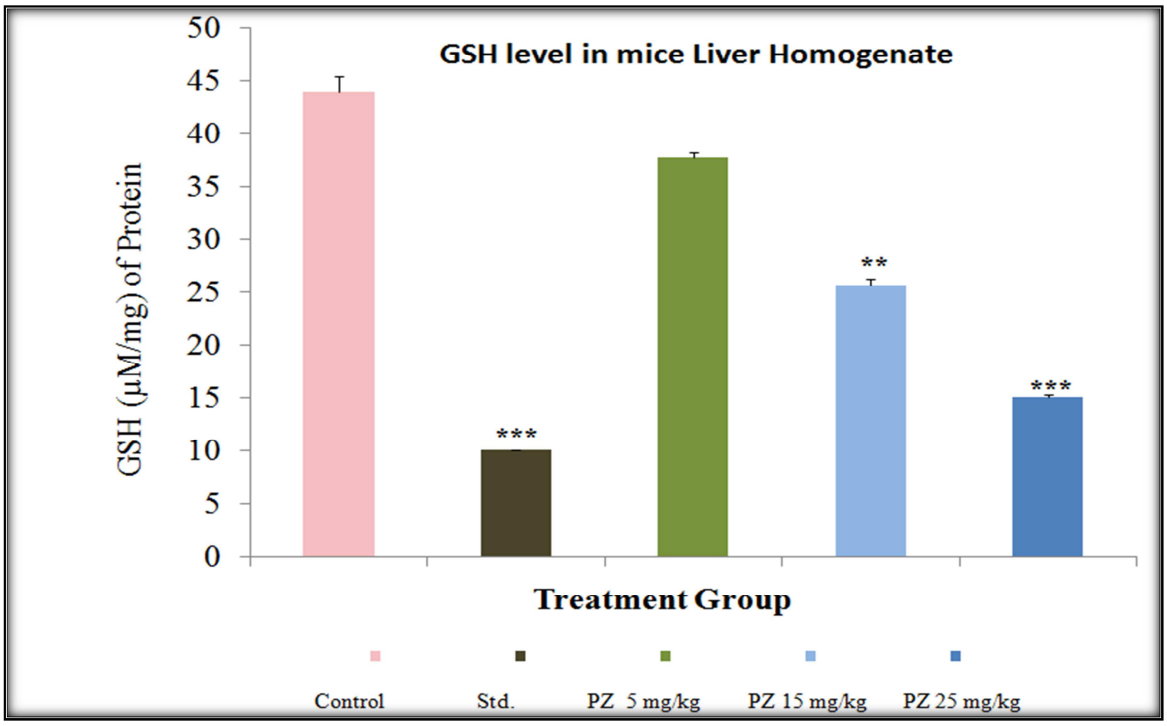

Figure 3 : GSH Level in Liver Homogenate in the Treated Groups
All values are expressed as Mean ±SEM (n=6/group)
$^{**}p < 0.01$ and $^{***}p < 0.001$ indicate level of statistical significance difference in comparison with control group.

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

Determination of PCE/PCE + NCE ratio in CP treated mice showed a pronounced cytotoxic effect of CP on bone marrow proliferation. The results are given in Table 3 for peripheral blood and in Table 4 for bone marrow. There was significant decrease in the PCE/PCE + NCE ratio both in bone marrow and peripheral blood in the PZ treated groups (at dose 25 mg/kg and 15 mg/kg) than that of control group ($p < 0.001$), while that at dose 5 mg/kg was comparable to that of control.

Table 3: Effect of PZ and CP Treatment on Percentage (%) of MN in Mice Peripheral Blood

Groups↓	MN %	PCE/(PCE + NCE)%
Control	0.18 ± 0.03	33.67 ± 0.50
Std (CP)	3.42 ± 0.06***	19.00 ± 0.29***
PZ (5 mg/kg)	0.23 ± 0.03	31.42 ± 0.42
PZ (15 mg/kg)	1.58 ± 0.03**	27.56 ± 0.37**
PZ (25 mg/kg)	2.68 ± 0.04***	23.67 ± 0.25***

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

***p < 0.01 and ***p < 0.001 indicate level of statistical significance difference in comparison with control group.*

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

Table 4 : Effect of PZ and CP Treatment on Percentage (%) of MN in Mice Bone Marrow

Groups↓	MN %	PCE/(PCE + NCE)%
Control	0.61 ± 0.10	60.09 ± 0.80
Std (CP)	5.56 ± 0.16***	33.63 ± 1.58***
PZ (5 mg/kg)	1.05 ± 0.10	56.60 ± 0.77
PZ (15 mg/kg)	1.89 ± 0.11**	46.40 ± 0.32**
PZ (25 mg/kg)	3.67 ± 0.17***	38.62 ± 0.36***

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

***p < 0.01 and ***p < 0.001 indicate level of statistical significance difference in comparison with control group.*

Effects of PZ on Chromosomal Damage in the Bone Marrow

Bone marrow CA assay is widely used to assess the clastogenic activity of chemicals. PZ treatment induced centromeric separations and chromatid gaps in Table 5. Different types of structural and numerical aberrations were observed. PZ treatment significantly increased the number of structural and numerical aberrations at higher doses i.e. 1 mg ($P < 0.01$) and 0.75 mg ($p < 0.05$) and Std. (CP) < 0.001 in comparison to the control group. The total percentage of these aberrations was found to increase in the treated group. This indicates that drug Prazosin damages the DNA.

Table 5 : Effect of PZ and CP Treatment on Chromosomal Aberration Assay on Mice Bone Marrow

Parameters → Groups↓	Structural aberrations					Gaps	Numerical aberrations		
	Ctb	Csb	Cms	other	Total (%)		pol	end	Total (%)
Control	0	0	1	0	0.5	1	0	0	0.5
Std (CP 30 mg/kg)	12***	9***	11***	10***	16***	10***	10***	13***	16.5
PZ (5 mg/kg)	0	1	0	1	1	1	1	0	1
PZ (15 mg/kg)	4	3	3	3	6.5**	3	3*	4*	5
PZ (25 mg/kg)	7**	5***	6**	6***	12***	5*	6**	8**	8.5

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

***p < 0.01 and ***p < 0.001 indicate level of statistical significance difference in comparison with control group.*

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

Quantitation of Fragmented DNA by Spectrophotometry

DNA fragmentation was quantified spectrophotometrically and significant increase in the percentage of fragmented DNA was observed in a dose-dependent manner in Fig 4.

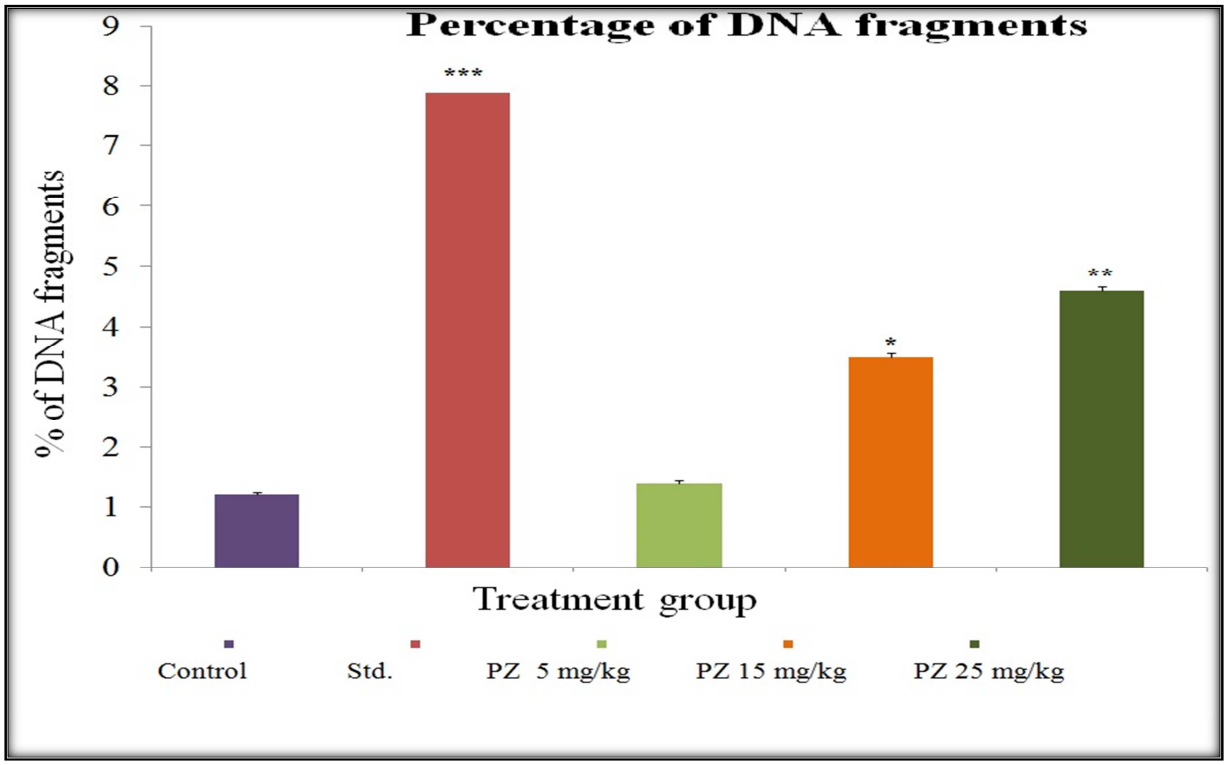

Figure 4 : Percentage of DNA Fragments in Liver Homogenate
All values are expressed as Mean ± SEM (n = 6/group)
$^{**}p < 0.01$ and $^{***}p < 0.001$ indicate level of statistical significance difference in comparison with control group

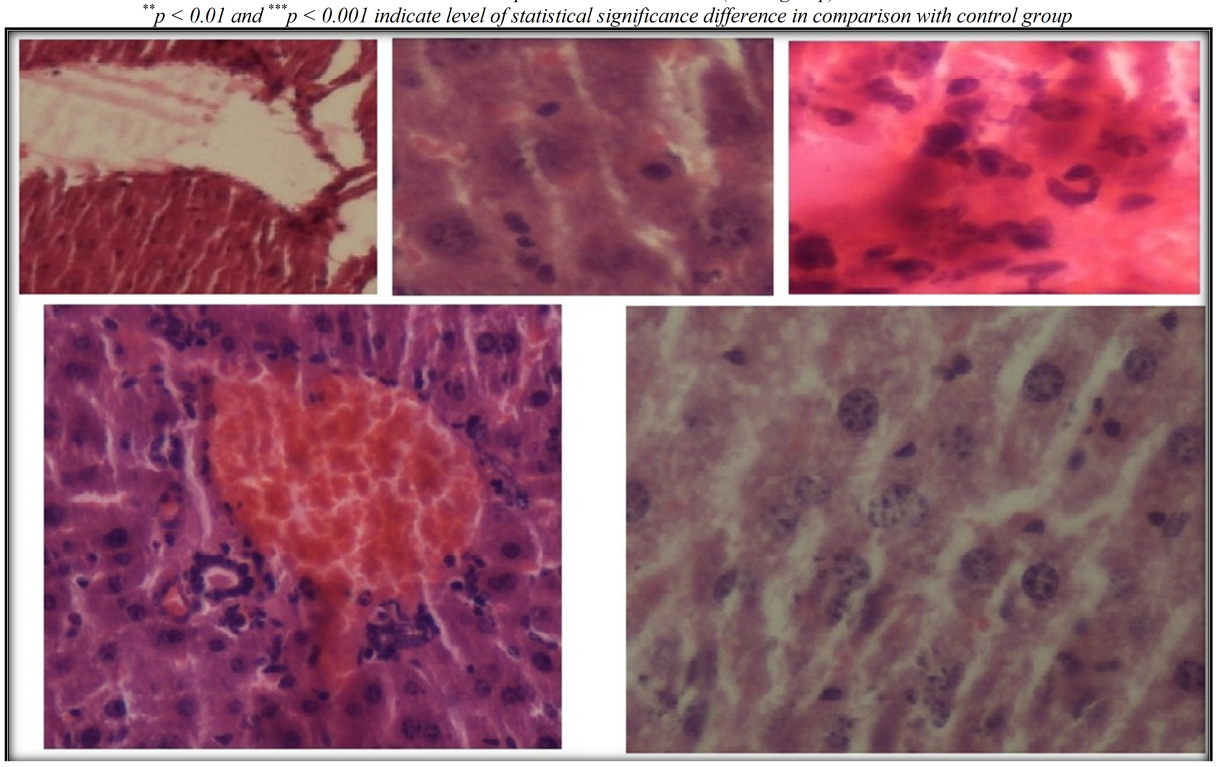

Figure 5 : Microscopic Photographs of Formation of Different Types of Chromosomal Aberrations in Mice Bone Marrow After Treatment with PZ and CP. Arrows Indicate the Portal Triad in Section A and C, Nuclear Damage in Section B, D and E
A: *Control* ***B:*** *Std. (CP 30 mg/kg)* ***C:*** *PZ (5mg/kg)* ***D:*** *PZ (15 mg/kg)* ***E:*** *PZ (25 mg/kg)*

Histological Examination of Liver Sections

The histopathological analysis of control liver section shows pinkish portal triad and central vein, hepatic venules. While the CP treated liver section shows hepatic necrosis, inflammation, congested venules, degenerated changes, karyolysis, large and small nucleus. PZ treated lowest dose (5 mg/kg) also shows pinkish portal triad and hepatic venules similar to control liver section. PZ treated (15 mg/kg) shows lesser degree of edema, cytoplasmic borders are smuggy, karyolysis absent, inflammatory cells present. And the PZ treated highest dose (25 mg/kg) liver section shows fluid filled within cells, karyolysis, nuclear and cytoplasmic edema, focal inflammation, all these findings are similar to that observed in the CP treated liver section. It can be concluded that drug PZ at the highest dose shows the toxicity in Fig 5.

Haemocrit analysis

Results are shown in Table 6.

Table 6 : Effect of PZ Treatment on Haemocrit Parameters

Groups↓	RBC (Million/Cmm)	TLC (/Cmm)	PC (lacs/Cmm)
Control	3.30	5,110	0.77
PZ (0.5 mg)	3.20	5,000	0.75
PZ (0.75 mg)	2.25	3,600	0.65
PZ (1mg)	1.44	2,700	0.50

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

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

DISCUSSION

In the present study, it is observed that Prazosin (PZ) exerts genotoxic and cytotoxic effects in the liver and bone marrow cells of mice. Statistically significant decrease in body weight, food intake and liver weight, in the treatment groups indicate systemic toxicity of Prazosin (PZ) in experimental animals, when compared to the control group. Prazosin as a selective α_1-adrenergic receptor antagonist used to treat hypertension. Its metabolism is primarily hepatic. According to oral bioavailability of prazosin ranges from 43.5 to 69.3% (mean 56.9%). Prazosin is highly (92 to 97%) bound to human plasma proteins (albumin and alpha 1-acid glycoprotein) and the extent of binding is independent of the plasma concentration of the drug in the range of 20 - 150 ng/ml [17]. In vitro investigations have revealed additional metabolic transformations of prazosin and have shown the potential of prazosin to undergo bioactivation through metabolism of the furan ring to a reactive intermediate [18]. Scientists have also reported in vivo metabolic route of prazosin consists of 6-O- and 7-O-demethylation followed by glucuronidation, with 6-hydroxy-prazosin glucuronide, the major metabolite. Other routes of metabolism include hydrolysis of the amide linkage to yield 2-(1-piperazinyl)-4-amino-6,7-dimethoxyquinazoline (N-desfuranoyl prazosin) and to a lesser extent, piperazine ring opening and N-dealkylation to give dimethoxyquinazoline-2,4-diamine (DQ) [19]. The metabolism of prazosin in humans has not been investigated extensively, and only N-desfuranoyl prazosin has been identified as a metabolite in humans [20]. Organ-specific toxicity of PZ can arise because of its bioactivation to chemically reactive metabolites, which can irreversibly bind to the tissue macromolecules [21].

Further, it is considered that the generation of reactive oxygen species (ROS) as one of the most important factors in the perturbation of cellular homeostasis and DNA damage. ROS oxidizes cellular fatty acids to form lipid peroxides. Electron microscopy study revealed the centrilobular hepatic necrosis in mice is attributed to vacuolation, loss of microvilli, and terminal hydropic degeneration.

Decrease in hepatic GSH is the reflective index of oxidant responses to hepatic necrosis rather than thiol oxidation mechanism involved in mediating the injury. After the 14 days of treatment with higher test doses of Prazosin (15 mg/kg and 25 mg/kg), lipid peroxidation product, i.e. MDA, levels were increased significantly in mice liver as compared to that of standard (Cyclophosphamide) treated group. Rise in MDA could be due to increased ROS generation, due to the excessive oxidative damage. These reactive oxygen species in turn can oxidize many other important biomolecules, including membrane lipids. Also, there was a significant decrease in the levels of reduced glutathione (GSH), in mice liver when treated with higher test doses of Prazosin (15 mg/kg and 25 mg/kg). PZ exerts genotoxic and cytotoxic effect in the hepatocytes. Further, it is claimed that the hepatotoxic injury is attributed to the mitochondrial compartmentalized oxidant effect of PZ. The mechanism of PZ-induced genotoxicity is well characterized in in-vivo test systems.

Metaphase chromosome analysis revealed that PZ induce structural chromosome aberrations. Many metaphases with clear centromeric separations, gaps, chromosomal breakage, chromatid breakage and numerical abnormalities like polyploidy were observed. Considering the previous report that chromosomal aberrations are caused by secondary mechanisms associated with cytotoxicity, it seems probable that the chromosomal aberrations induced by PZ represent an outcome of an indirect activity associated with cytotoxicity rather than its direct action on DNA. Aberrations like centromeric separations and gaps might be attributed to the compound's more affinity toward the protein moiety of the DNA, rather than the double strand breaks. Do not consider gaps in the statistical analysis because of their controversial genetic significance. Furthermore, in the 14 days single dose study, the effects produced at the highest dose (25 mg/kg) was more than the immediate lower dose (15 mg/kg), which might be attributed either to the perturbations in the cell cycle or to the generation of target organ-specific cytotoxicity of the compound. Further, it has been reported that PZ induce dose- and time-dependent cytotoxicity and irreversible binding to the macromolecules of mouse and rat hepatocytes.

PZ was rated as positive in the in vivo chromosomal aberration test because the frequency of structural abnormalities was over 10% at cytotoxic i.e. two higher doses of PZ (15 and 25 mg/kg). Also PZ tested positive in the mouse bone marrow and peripheral blood micro nucleus (PBMN) test and in the DNA fragmentation assay, suggesting that the PZ damages DNA in vivo at higher dose concentrations. The decrease was found in PCE/ (PCE + NCE) % in the PZ treated groups in both bone marrow and peripheral blood. The result of bone marrow micro nucleus (BMMN) was around 22% in the highest dose (25 mg/kg) while it was 14% in the medium dose (15 mg/kg) in comparison to control group, while in PBMN it was 16% decrease in comparison to control group respectively. In the standard i.e. CP treated group (30 mg/kg) the reduction was highest both in PBMN and BMMN. No decrease was found in the lowest dose (5 mg/kg) of PZ. Increase in MN % was observed in both PBMN and BMMN in comparison to the control group. These data indicates significant growth suppression in erythrocytes means that the drug PZ was adequately exposed to the target tissue (bone marrow) in the experiment. The increase in % of DNA fragments with higher test doses of Prazosin (0.75mg/kg and 1mg/kg) in mice liver homogenate indicates the DNA damage. Histopathological analysis also supports the other observations to conclude that PZ is genotoxic and cytotoxic at two higher doses as the liver section shows karyolysis and cytoplasmic edema formation.

Programmed cell death, i.e., apoptosis plays a key role in the maintenance of the steady state in continuously renewing tissues. PZ produced significant increase in the fragmented DNA in the two higher doses when compared to the control. According to a post marketing survey and prazosin might cause low platelet count (may cause bleeding problems). This drug may also cause the following symptoms those are related to low platelet count: aplastic anemia, low counts of all blood cells including red and white blood cells, and platelets.

Similar findings have been reported in the present study as the haemocrit analysis reported decrease in the leucocytes and platelet count after 14 days of drug administration hence drug shows effect similar to that of anti-platelet drug. PZ decreases the permeability of blood vessels in turn decreases the aggregation of platelets.

CONCLUSION

In summary, the present study provides evidence that Prazosin induced significant genotoxic effects in mice at its equivalent hepatotoxic dose level. The positive results in the MN assay might be attributed to the DNA strand breaks resulted by PZ induced oxidative stress, which was sufficient to induce structural aberrations in the metaphase analysis and increase % of DNA fragments. Presence of karyolysis, nuclear and cytoplasmic edema in the histopathological analysis further supports that the DNA damage has occurred in liver. Further works at molecular level can help to better understand its exact mechanisms of toxicity and to take further regulatory decision on its safety issues, because it belongs to an important therapeutic group having huge clinical use.

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