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Preclinical Toxicity Assessment of Polyphenols-Based Standardized Extract of *Cinnamomum zeylanicum Bark*

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Abstract

Cinnamon (*Cinnamomum zeylanicum*) bark is used as a spice in culinary practices and has been documented for health and medicinal benefits. Polyphenol (PP) is the major bioactive constituent of cinnamon bark. However, acceptable and safe dose levels and toxicity information of oral exposure to PP-based standardized cinnamon bark extract (PP-CZ) are crucial for safe human consumption. To evaluate PP-CZ for acute oral, subchronic oral toxicities *in vivo*, mutagenicity *in vitro* and genotoxic potential *in vitro*. The effects of oral treatment with single- and 90-days repeated dose were evaluated in rates as per OECD Test 423 and 408, respectively. Effects on body weight, food and water intake, organ weight, hematology, biochemistry, and histology were recorded. Mutagenicity and genotoxicity were evaluated using Ames (OECD No. 471) and chromosomal aberrations (OECD Test. 473) tests. A single oral dose of PP-CZ did not cause death or treatment-related toxic effects, indicating a "median lethal dose" > 2,000 mg/kg. In addition, a subchronic dosage (500 mg/kg/day, 90 days) was found safe in rats, suggesting "no observed adverse effect level" (NOAEL) of 500 mg/kg and "Human Equivalent Dose" (HED) of 4.8 g/day. Furthermore, the absence of mutagenicity or genotoxicity of PP-CZ was observed during *in vitro* tests. PP-CZ showed a robust safety profile without mutagenicity or genotoxicity in rats.

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Introduction

The need for safety evaluations of natural medical products has been the subject of discussion and reviews.[1-4] Numerous natural substances are included as bioactive ingredients in herbal medicine, functional foods, and nutritional supplements.[5,6] In addition, excessive intake of natural products may raise safety concerns because of their accumulation in the human body.[7] Thus, each component originating from plants must be assessed for

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acceptable and safe dose levels using internationally recognized guidelines.[8] Such data can serve as a foundation for risk assessment of plant-derived medicinal and healthcare products for human consumption.

The cinnamon (*Cinnamomum zeylanicum* Syn. *C. verum*) is a well-known ingredient for food reparation as flavor,[9] seasoning[10] and aroma.[11] In addition to its culinary use as a spice and flavor, cinnamon bark has many medicinal properties in Indian[12] and Chinese[13] traditional systems. In modern literature, many pharmacological activities of cinnamon bark include nutraceutical,[14] medicinal properties against diabetes.[15] cognitive disorders,[16] cardiovascular disorders,[17] liver disorders[18] and anti-inflammatory.[18] Cinnamon bark has many bioactive phytochemical constituents, including volatile oil, cinnamaldehyde, cinnamic acid, tannins, mucilage, terpenes, and polyphenols.[19] However, the toxicological data on cinnamon bark-based products, especially standardized extracts, are limited[20,21] or reported as a diet, or crude bark extract.[22] Furthermore, the safety of crude extract[23] and few constituents of cinnamon bark, such as cinnamon bark oil and cinnamaldehyde, have been reported in animals.[24, 25] However, evidence of the safety or toxicity of standardized extract of cinnamon bark as per internationally recognized and reliable guidelines, such as "Organization for Economic Cooperation and Development" (OECD) guidelines,[26] is not available.

The broad pharmacological profile is attributed mainly to cinnamon bark's polyphenols (PP) content.[27-29] The most prominent polyphenols of cinnamon bark are oligomeric procyanidins (OPC).[30,31] Cinnamon bark comprises type-A procyanidin PPs, including pentameric, tetrameric, and trimeric polyphenols.[32] The PP-based standardized cinnamon bark extract (PP-CZ) has evidence of efficacy against immune-inflammatory disorders as reported in animal models of allergic rhinitis,[33] asthma,[34] arthritis[35,36] and clinical studies.[37,38]

Toxicological evidence of cinnamon bark has been limited to crude extracts[39] or powder[40] in which the exact dose of bioactive compounds cannot be estimated. Furthermore, the absence of mutagenicity in OPCs, such as procyanidin B4 and procyanidin A2, isolated from various natural sources has been reported.[41] However, toxicological information on standardized cinnamon bark extract, such as PP-CZ, in compliance with international regulatory guidelines, is essential to estimate safe human exposure levels and risk assessment in clinical scenarios. Therefore, the present study aimed to determine the preclinical safety of PP-CZ *in vivo* and *in vitro*, as per OECD guidelines.

Materials and methods

Materials

This study was performed with protocol approval that complied with the ethical norms of animal experiments in India.[42] Acute and subchronic toxicity studies were performed on Sprague-Dawley rats of both sexes (Sprague Dawley) maintained in an animal house at ambient humidity, temperature, and light/dark cycle (12 h) with feed and filtered water ad *libitum*.

Indus Biotech Limited (Pune, India) provided the PP-CZ powder. PP-CZ (also known as IND02) is a standardized cinnamon bark extract, standardized to 60.31% polyphenol content by Folin-Ciocalteu assay.[43] A fresh suspension in distilled water was prepared daily and orally (gavage) administered in a volume of 10 ml/kg.

Acute Oral Toxicity (AOT)

OECD guideline No. 423 was used to evaluate the AOT of PP-CZ.44 Two groups of five rats per sex (125-150 g, 6-8 weeks) were made, namely G1 (Vehicle Control, VC) and G2 (PP-CZ treated). A single dose of either vehicle (distilled water) or PP-CZ (was orally administered to G1 and G2 rats, at 0 and 2000 mg/kg respectively. Mortalities, Weights, and morbidities were tracked and noted for 14 days duration.

Subchronic Toxicity

The OECD guideline No. 408 was used to assess subchronic repeated dose toxicity of PP-CZ on 90-days of treatment.[8] The 80 males and 80 females (age: 4-6 weeks, weights: 90-120 g) were randomly divided into 15 rats/group per sex (G1 to G4)) and 10 rats per sex (G1R and G4R). The limit dose was 1000 mg/kg/day as per OECD guidelines.[8] Lower doses were decided as one-fourth (250) and one-half (500) of the limit dose (1000) as per the guidance.[8] All treatments were administered to rats orally once daily. The treatments were as follows: Group G1 was

VC, treated with distilled water for 90 days; G2-G4 were treatment groups (PP-CZ-250, PP-CZ-500, PP-CZ-1000) and treated with oral treatment of PP-CZ at doses 250 mg/kg/day, 500 mg/kg/day, and 1000 mg/kg/day for 90 consecutive days, respectively. Reversal groups (G1R and G4R) - VC-R and PP-CZ-1000-R - received vehicle, PP-CZ (1000 mg/kg/day), and vehicle for 90 and 28 days (Day 91-119) respectively.

Daily observations of mortality and clinical signs of the rats were conducted for 90 days (G1–G4) or 119 days (G1R and G4R). Ophthalmoscopy was performed on the eyes of rats in all groups prior to (day-0) and last day of treatment, on the 91st day (G1-G4) and 119th day of the study for G1R and G4R, respectively. Body weight and food intake were measured weekly.

Functional observations were performed by grading the ear (auditory), eye (visual), and proprioceptive stimulus reactivity. Urine and blood samples were collected before scheduled necropsy. Urinalysis parameters included color, volume, bilirubin, ketones, glucose, occult blood, nitrite,specific gravity, pH, appearance, and protein.

After the scheduled treatments, euthanasia and necropsy were performed on the rats, except for those in the recovery group. Organs such as the liver, kidneys, testes, uterus, thymus, brain, lungs, adrenal glands, epididymis, ovaries, spleen, and heart were isolated for relative organ weight measurements. Histopathology of the organs was also conducted as needed by OECD guidelines. Davidson's fluid was used to preserve the eyes and testes and 10% formalin was used to preserve other organs until hematoxylin and eosin staining. Histopathological examinations were performed using LABCAT software (Innovative Programing Associate, Inc., Princeton, USA).

Mutagenicity

Mutagenicity evaluations were performed using the "bacterial reverse mutation Test No" (also called the AMES test) according to OECD guidelines, Test No: 471 using histidine auxotrophic bacterial strains (TA97a, TA98, TA100, TA1535, and TA102) of *Salmonella typhimurium* [45,46] obtained from Bruce Ames Laboratory (Berkeley, USA). A concentration-finding study (conc. One to 5000 µg/plate) followed by a final mutagenicity study with and without metabolic activation was performed as per guideline using the post-mitochondrial fraction (S9 fraction). The histidine revertant colonies, as prototrophs, were scored on plates with the mean number compared. A concentration-dependent increase was checked as the number per plate or a repeatable increase in at least one colony or concentration was considered to indicate positive mutagenicity.

Genotoxicity

Genotoxicity of PP-CZ 25, 12.5 and 6.25 mg/culture was assessed using an "mammalian chromosome aberration (CA) test *in vitro*" (Test No. 473) [47] on 24 h exposure. Separate tests were conducted using distilled water (solvent control) and EMS (positive mutagens). Positive genotoxicity was indicated by a dose-dependent increase in structural chromosomal aberrations at all concentrations.

Statistical Analysis

The data are presented as mean ± standard deviation (SD) and were analyzed using SPSS v.16.0 (SPSS Inc., Chicago, USA). Differences were considered statistically significant at $P < 0.05$. The data for each parameter of the in vivo test were analyzed using Bartlett's homogeneity test, followed by an unpaired t-test. The chromosomal aberration data are presented as the percentage of aberrated cells and frequency (number per cell) and were analyzed using Student's t-test and Cochran-Armitage test, respectively.

Results

AOT

All rats (both sexes) treated with PP-CZ (2000 mg/kg) survived without toxic effects, and so suggested as "median lethal dose" (LD_{50}). No body weight gain was observed. Gross pathology and microscopic examination of the rats indicated no abnormalities due to treatment.

Subchronic Toxicity

Survival, Clinical Signs, Food Consumption and Body Weights

No death, abnormalities (eye or functional), or clinical signs of toxicities were present during treatments, recovery periods or necropsy. Body weight and food consumption of PP-CZ-treated rats were not significantly different between the groups.

Table 1: Hematology - Male rats (Subchronic toxicity study)

Parameter	VC	PP-CZ-250	PP-CZ -500	PP-CZ -1000	VC-R	PP-CZ -1000-R
Reticulocytes (%)	1.66 ± 0.43	1.55 ± 0.45	1.59 ± 0.45	1.53 ± 0.45	1.56 ± 0.47	1.56 ± 0.35
HCT (%)	42.06 ± 2.51	41.81 ± 1.85	41.91 ± 1.18	44.69 ± 3.47*	45.32 ± 6.74	49.80 ± 5.46
RBC (x106 /µL)	8.30 ± 0.50	8.19 ± 0.47	8.30 ± 0.36	8.98 ± 0.60**	9.05 ± 1.28	9.41 ± 1.06
MCV (mm^3)	50.70 ± 2.35	51.11 ± 2.31	50.55 ± 1.71	49.75 ± 1.66	49.98 ± 1.43	52.98 ±1.07**
MCH (pg)	17.48 ± 0.76	17.66 ± 0.82	17.39 ± 0.75	17.05 ± 0.68	17.08 ± 0.56	18.22 ± 0.25**
MCHC (%)	34.48 ± 0.51	34.53 ± 0.29	34.37 ± 0.43	34.31 ± 0.59	34.16 ± 0.30	34.40 ± 0.41
Hb (g%)	14.49 ± 0.85	14.44 ± 0.69	14.41 ± 0.41	15.32 ± 1.18*	15.50 ± 2.41	17.12 ± 2.01
Platelets (x 10^3 /µL)	427.47 ± 98.13	411.47 ± 50.49	408.93 ± 48.83	399.73 ± 91.53	338.60 ± 72.60	350.80 ± 84.02
Pt (sec)	14.80 ± 3.43	13.80 ± 3.65	14.67 ± 4.20	15.67 ± 3.20	14.00 ± 2.25	15.00 ± 3.39
TLC (x 10^3 /µL)	13.72 ± 4.98	12.82 ± 3.40	12.58 ± 2.35	13.18 ± 3.47	9.80 ± 3.42	8.22 ± 1.08
Differential Leukocyte count						
N (%)	20.67 ± 3.83	21.73 ± 3.49	21.47 ± 3.40	21.53 ± 2.95	21.00 ± 4.42	21.00 ± 3.39
M (%)	2.13 ± 0.83	2.27 ± 0.70	2.07 ± 0.80	2.27 ± 0.70	2.20 ± 0.84	2.00 ± 0.71
L (%)	76.00 ± 3.27	75.00 ± 3.64	75.40 ± 3.18	75.20 ± 2.73	75.80 ± 3.70	75.80 ± 3.11
E (%)	1.20 ± 0.77	1.00 ± 0.85	1.07 ± 0.88	1.00 ± 0.85	1.00 ± 0.71	1.20 ± 0.84

Data as Mean ± Standard Deviation, * P < 0.05, ** P < 0.01 (v/s VC, unpaired t test)

Table 2: Hematology - Female rats (Subchronic toxicity study)

Parameter	VC	PP-CZ-250	PP-CZ -500	PP-CZ -1000	VC-R	PP-CZ -1000-R
Reticulocytes (%)	1.60 ± 0.42	1.50 ± 0.45	1.61 ± 0.47	1.51 ± 0.52	1.64 ± 0.30	1.54 ± 0.46
HCT (%)	40.19 ± 1.89	47.81 ± 8.29**	49.07 ± 6.24**	42.06 ± 1.97	43.62 ± 2.19	40.62 ± 1.90*
RBC (x106 /µL)	7.79 ± 0.43	9.22 ± 1.67**	9.40 ± 1.10**	8.02 ± 0.31	8.21 ± 0.32	7.59 ± 0.60*
MCV (mm^3)	51.59 ± 1.34	51.91 ± 1.33	52.21 ± 2.00	52.43 ± 1.90	53.12 ± 1.55	53.64 ± 2.17
MCH (pg)	17.99 ± 0.60	18.25 ± 0.48	18.15 ± 0.69	18.07 ± 0.3	18.20 ± 0.21	18.82 ± 0.75*
MCHC (%)	34.83 ± 0.57	35.15 ± 0.30	34.77 ± 0.31	34.45 ± 0.57	34.28 ± 0.61	35.10 ± 0.23**
Hb (g%)	14.00 ± 0.70	16.78 ± 2.90**	17.05 ± 2.13**	14.50 ± 0.90	14.96 ± 0.59	14.28 ± 0.57*
Platelets (x 10^3 /µL)	405.27 ± 65.41	327.60 ± 108.69*	356.27 ± 114.12	387.93 ± 53.36	404.20 ± 60.49	394.00 ± 40.90
Pt (sec)	15.00 ± 3.30	15.00 ± 3.85	15.40 ± 3.25	15.07 ± 3.33	15.00 ± 2.74	15.40 ± 3.36
TLC (x 10^3 /µL)	10.01 ± 2.92	9.87 ± 3.76	11.55 ± 2.96	10.52 ± 3.01	7.02 ± 0.77	7.18 ± 1.67
Differential Leukocyte count						
L (%)	75.40 ± 3.50	75.73 ± 3.58	75.87 ± 3.36	75.27 ± 2.55	75.80 ± 3.63	75.60 ± 4.72
N (%)	21.07 ± 3.65	21.00 ± 4.17	21.00 ± 3.89	21.40 ± 3.04	20.80 ± 3.96	21.20 ± 4.38
M (%)	2.27 ± 0.70	2.33 ± 0.82	2.07 ± 0.80	2.27 ± 0.96	2.20 ±	2.00 ±
E (%)	1.27 ± 0.80	0.93 ± 0.88	1.07 ± 0.80	1.07 ± 0.80	1.20 ± 0.84	1.20 ± 0.84
Pt (sec)	15.00 ± 3.30	15.00 ± 3.85	15.40 ± 3.25	15.07 ± 3.33	15.00 ± 2.74	15.40 ± 3.36

Data as Mean ± Standard Deviation, * P < 0.05, ** P < 0.01 (v/s respective VC, unpaired t test)

Hematology

The results of the hematological investigations of male (Table 1) and female (Table 2) rats are presented. All values from hematological observations were within normal reference ranges, with few statistically significant changes (vs. respective control groups) in the values of a few parameters. However, no cause-and-effect correlation could be established relating to dose, treatment duration (treatment/reversal), or sex. For example, PP-CZ-1000 significantly increased Hb, RBC, and HCT in males (vs. VC). In recovery group, PP-CZ-1000R group, significant decrease in Hb, RBC, and HCT levels (vs. VC-R) in females was found. However, these hematological parameters remained unaffected in the recovery group of male rats only. MCV values for the PP-CZ-1000 group (vs. VC-R) of male rats showed significant increase without such changes in females. However, a significant increase in MCHC values was observed in females but not in male rats. The MCH values for PP-CZ-1000 of rats of both sexes showed a significant increase (v/s VC-R).

Blood Biochemistry and Urine Analysis

Biochemical measurements (liver and kidney function, serum electrolyte balance, and metabolic function parameters) were performed. All values were within the normal limits for both sexes, except for a few statistically significant changes (vs. the respective control groups). However, no cause-and-effect correlation was found between sex, dose, and duration of observation (treatment/reversal) in the PP-CZ-250 and PP-CZ-500 groups.

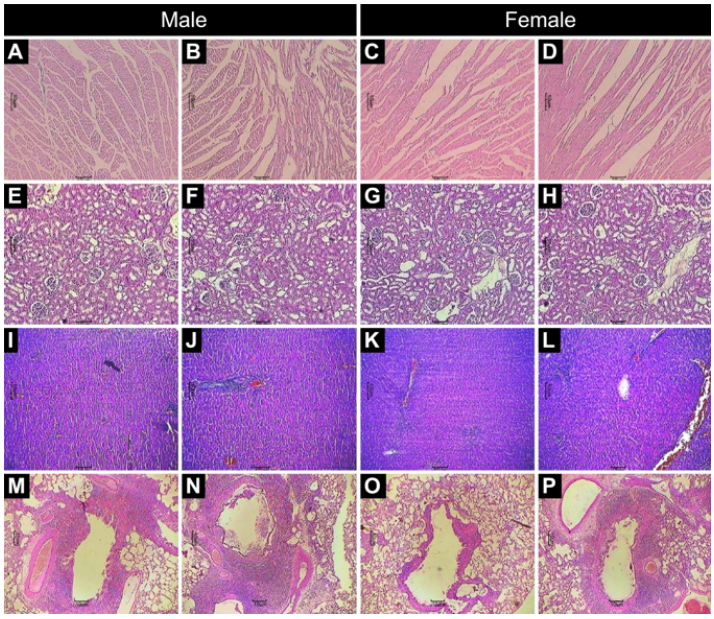

Fig. 1: Effects of PP-CZ on histological findings from a 90- days repeated dose toxicity study. Figure showing sections of representative organs: heart (A–D), kidney (E–H), liver (I–L) and lung (M–P) tissue in rats and respective groups: VC (A, E, I and M), PP-CZ-1000 (B, F, J and N), VC-R (C, G, K and O) and PP-CZ-1000-R (D, H, L and P) (H&E stain) at 40X

Few biochemical parameters in PP-CZ-1000 treated male rats (ALT and ALP), female rats (ALT, ALP, total proteins, and glucose), and PP-CZ-1000R in females (ALT) showed significant changes, but within normal biological ranges. In female rats, some electrolyte levels (Ca, Cl, and Na) showed significant differences with respective VC groups. In contrast, males did not show such changes in their serum electrolyte levels, except for Ca and Na in PP-CZ-100-R group. Metabolic function parameters, such as cholesterol, triglycerides, and LDH, remained unaffected, except for PP-CZ-500 in female rats (vs. VC). Urinalysis did not show abnormalities in PP-CZ-treated groups.

Gross Pathology and Histopathology

During necropsy, no pathological changes attributable to the treatment were observed in the PP-CZ or VC

groups. The relative weights of none of the organs, either PP-CZ-treated or VC in male or female rats, remained unaffected, with no significant changes between the groups, except in heart of PP-CZ-250-treated male rats (decreased) and kidneys in PP-CZ-1000 treated female rats (increased). However, the relative weights (relative) of all organs in the PP-CZ-1000-R recovery group remained unaffected (no statistical difference). Histology of the organ sections had no treatment attributable abnormalities and so considered incidental. The histological findings from photomicrographs of representative vital organs, namely lung, heart, kidney, and liver tissues in rats are presented as Figure 1

According to the subchronic toxicity evaluation of PP-CZ in this study, dose of 500 mg/kg/day for 90 days was recommended as the "no observed adverse effect level" (NOAEL) for subchronic oral treatment in rats.

Mutagenicity

PP-CZ (5000 µg/plate and lower) caused no significant differences in bacterial background lawn during the two mutagenicity experiments, with no statistical significance in revertant colony counts, regardless of metabolic activation. In contrast, the positive control group showed a significant increase. Spontaneous reversion in the controls was within the historical range. Therefore, PP-CZ was confirmed to be a non-inducer of gene mutations and is not mutagenic.

Genotoxicity

The genotoxicity study data for various culture concentrations, solvent controls, and negative controls in both experiments confirmed the absence of mitotic activity induction at 25 mg/culture. Chromosomal break analysis, chromosome aberrations (number), and percentage of aberrated cells were not significantly different between the groups during both experiments (4 h and 24 h), with or without metabolic activation. Because aberrations induced by PP-CZ were exclusively chromatid-type, polyploidy or endoreduplication was not generated.

Discussion

Regulatory agencies worldwide have issued regulatory guidelines emphasizing safety or toxicological evaluations and reporting for different types of products, such as drugs, cosmetics, dietary supplements, and complementary medicines.[6,48-51] Most of these guidelines recommend toxicological evaluations using suitable animals to mimic most human clinicopathological features. The OECD guidelines are internationally recognized criteria for evaluating the safety or toxicity of chemicals, including natural products.[26] In the present study, the toxicity of PP-CZ on acute and subchronic oral exposure (repeated dose for 90 days) to rats, with its mutagenicity and genotoxicity assessed using the corresponding OECD guidelines.

In AOT, the substance is considered non-toxic in the absence of death and normal clinical signs at an acute dose of 2000 mg/kg.[52] In the present study, PP-CZ did not exhibit any toxicity and lethality within 14 days in males or females at LD_{50} (>2000 mg/kg).

Repeated-dose toxicity studies in rodents are recommended to evaluate the cumulative adverse effects of test compounds on repeated exposure over a limited period.[8] The present subchronic toxicity evaluation of PP-CZ was conducted using three dose levels, starting from 250 mg/kg/day with the next dose double of lower, once a day, for 90 days, as suggested by the OECD guidelines.[53] During which 90-day administration of PP-CZ did not result in any mortality, clinical abnormalities, changes in animal behavior, food consumption, water consumption,or weights in the tested dose range (250 to 1000 mg/kg).

A repeated-dose toxicity study can be a major consideration for estimating the NOAEL.[54] The observation, such as increased body weight is conered favorable and decline in body weight and relative organ weights suggest adverse effects with an indicator of toxicity.[55] In the present study, subchronic oral administration of PP-CZ showed consistent weight gain and food consumption during the study period without a statistical difference compared with the VC group in both sexes. These results confirmed the earlier reports of the absence of body weight changes on consuming other polyphenolic food chain compounds, flavonoids, or proanthocyanidin-rich extract from grape fruits or seeds, respectively [56,57]

Hematological observations in animals have been reported to have a clinical correlation with changes in human blood parameters with a higher predictive

value.[58] A significant increase in Hb, RBCs, HCT, MCV, MCH, and MCHC was observed in PP-CZ-treated rats. However, all the values were normal.[59,60] These results can be attributed to hematopoiesis stimulation by procyanidins, as other plant-derived procyanidin polyphenols reported for inhibition of RBC hemolysis in the diabetic condition[61] and prevent a radiation-induced decline in RBC and Hb.[62]

Biochemical evaluation is an integral part of toxicological studies. Many natural products have been reported to cause significant liver toxicity [63-65] and kidneys.[66-68] Biochemical parameters such as AST, ALT, ALP, GGT, bilirubin, total protein, and albumin are considered hallmarks of liver function.[69] None of liver function parameters was found to be significant in PP-CZ-treated rats of either sex (vs. respective VC groups), except for an increase in ALT and AST in the PP-CZ-1000 group in this study. Furthermore, all values were within the normal physiological range with no pattern or trend with respect to sex or dose. Moreover, no significant alteration was recorded in the liver weights in any group. Histopathology of the liver samples had no changes, necrosis or damage to cell architecture of severe nature and so clinically insignificant.

Biochemical parameters such as urinary pH, CR, CK, and BUN are markers of kidney function.[70] All rats maintained normal values of urinary function parameters in both sexes. In addition, urinalysis and histological examination of the kidneys did not reveal any signs of severe toxicity. However, a mild degree of focal lymphocytic infiltration and necrosis was observed during histopathological examination of the kidney samples. Such changes have been reported as normal developmental processes in the kidneys of Sprague-Dawley rats [71] and, therefore, were not considered significant, toxicologically, or clinically. Some serum electrolyte parameter values showed significant changes in the PP-CZ-1000 and PP-CZ-1000-R groups in females, but not in males. However, all electrolytes levels were within the normal reference ranges.[59,60] The blood biochemical parameter values of FPG, cholesterol, triglycerides, and LDH are indicators of metabolic functions, which did not changed with PP-CZ-treated rats as compared to normal physiological reference ranges.[72]

Based on the present results, the NOAEL of PP-CZ after subchronic exposure was 500 mg/kg in rats. These results of safety of PP-CZ for subchronic exposure are supported by past reports of safety in mice on a cinnamon diet (safe at 1000 and 2500 mg/kg/day, 16 weeks exposure),[22] and water extract extracts of cinnamon bark (100 mg/kg/day, 3 months of exposure).[73]

Mutagenicity is a critical toxicological assessment that ensures the ability of any substance to induce mutations. Hence, the Ames test, developed in the 1970s, is routinely used to screen mutagens and has traditionally been utilized for mutagenicity screening.[45] It assumes that a substance that is mutagenic to the bacterium such as *Salmonella typhimurium* in with/without metabolic activation can pose a risk of cancer in humans.[74] In this study, a broad range of concentrations of PP-CZ (61.72–5000 µg/plate) with or without a metabolic activator did not display mutagenic activity. The number of histidine revertants in the negative and positive controls after PP-CZ exposure was within the acceptable range, implying a lack of mutagenicity potential. Several researchers have reported a lack of mutagenicity in cinnamon extracts, cinnamon bark oil, and cinnamaldehyde using the AMES test.[75,76] Our results provide additional evidence regarding the non-mutagenic nature of polyphenol-based standardized extracts from cinnamon bark.

Amongst the silent characteristics of cancer cells, including genetic instability, aberrant cell differentiation, and proliferation[77] and accelerated carcinogenesis.[77] Therefore, CA assay, which measures the effects of test compound exposure on genetic alterations (e.g., aberrations) in blood lymphocytes, has been used for genotoxicity and cancer assessment.[78] The CA assay is adependable and widely accepted for assessing genotoxicity risk.[78] This method is well regarded owing to its reliability in detecting chromosome aberrations, which are commonly observed in tumor suppressor loci and contribute to the process of carcinogenesis.[79] We used peripheral blood lymphocytes, in presence and absence of metabolic activation, in CA assay. Here, PP-CZ at 6.25, 12.5, and 25 µg/culture did not cause a significant increase aberration numbers with/

without metabolic activation, indicating an absence of genotoxicity.

In summary, the above results suggest the robust preclinical safety of PP-CZ in acute and subchronic toxicity without potential mutagenicity or genotoxicity. The safe human equivalent dose (HED) of PP-CZ as per US Food and Drug Administration[80] based on NOAEL (500 mg/kg) will be 4.8 g/day for oral consumption in humans, considering a weight of 60 kg. In the past, oral supplementation of PP-CZ as an adjuvant to chemotherapy was reported to reduce chemotherapy-induced side effects (weight loss and alopecia) in breast cancer patients at 1.2 g/day or 1200 mg/day (400 mg, thrice a day).[37] The safe HED (4.8 g /day) was found to be four times the clinical efficacy dose (1.2 g/day),[37] which indicates the robust safety of PP-CZ for clinical use. However, further research may be required for detailed risk assessment in special physiological conditions such as pregnancy, or long-term use as maintenance treatment in patients with chronic diseases, alone or as an adjuvant to existing therapy.

Conclusions

Acute or subchronic oral administration of PP-CZ to rats resulted in a robust safety profile without mutagenicity or genotoxicity. PP-CZ was found to have an oral LD_{50} < 2000 mg/kg (limit-dose), NOAEL = 500 mg/kg (HED=4.8 g/day) on subchronic administration.

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Conflict of Interest

The authors declare no conflicts of interest.

Author's contributions

Prasad Thakurdesai : Drafting the manuscript, Revising and approval of manuscript. Pallavi Deshpande: Revising and approval of manuscript. Sunil Bhaskaran: Conception, Design of study, Revising and approval of manuscript.

Data Availability Statement

Data related to this article can be obtained from the corresponding author upon reasonable request.

Ethics Approval Statement

All animal experiments were performed according to the guidelines issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with protocol approval at the Indian Institute of Toxicology, Pune, India (No.15/1999/CPCSEA).

Informed Consent Statement

Not applicable

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