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Assessment of Titanium Dioxide Nanoparticles (TiO_2-NPs) Induced Hepatotoxicity and Ameliorative Effects of *Cinnamomum cassia* in Sprague-Dawley Rats

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Abstract This study assessed the protective effects of *Cinnamomum cassia* (cinnamon) bark extract in rats exposed to titanium dioxide nanoparticles or titanium dioxide bulk salt. For in vivo evaluation of the ameliorative role of the cinnamon extract, the experimental groups were orally administered with the cinnamon extract at different dose levels (50 or 100 or 150 mg/kg bodyweight) along with the subcutaneous injections of 150 mg/kg bodyweight titanium dioxide nanoparticles or titanium dioxide bulk salt. The extract showed significant ameliorative role on the antioxidant system in response to elevated levels of titanium dioxide nanoparticles or titanium dioxide bulk salt-induced oxidative stress. It aided in the recovery of the antioxidant system as well as protective role in histological damages and some haematological parameters in the rat liver treated with titanium dioxide nanoparticles or titanium dioxide bulk salt.

Keywords TiO_2-NPs · TiO_2 bulk salt · Histopathology · Haematology · Sprague-Dawley rats

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Introduction

Titanium dioxide (TiO_2) is one of the most commonly used materials and is among the top five nanoparticles being synthesized [1]. It is being used in nanoform in various industrial fields such as paints, pigments, dyes, alloys, rubber, ceramics, chemical fibres, sunscreens and other cosmetics, tooth pastes, electronics, pharmaceutical preparations, industrial photocatalytic processes, personal care products, food additives, catalyst and metallurgy and in the environmental decontamination of air, soil and water [2–9]. Due to the extensive application of TiO_2 nanoparticles (NPs) in the industrial field and ongoing commercialization of nanotechnology products, the exposure of the human body intentionally or unintentionally to nanoparticles via several possible routes, including dermal penetration, inhalation, oral ingestion or intravenous injection, is possible and may continue to increase [3, 10]. Therefore, the analysis of its potential toxicity and distribution in the body as well as the remedial activity of natural and easily available antioxidants is essential. Toxic effects of titanium dioxide nanoparticles and titanium dioxide bulk salt in the liver and blood of male Sprague-Dawley rats have been assessed by different assays in our previous study [11], and a number of studies regarding the toxicity due to TiO_2 nanoparticles in other body parts in different organisms have been reviewed by Shakeel et al. [12].

Drugs from natural sources are used for treating various diseases since the ancient times [13]. Plants have always been an ideal source of drugs. Many of the drugs that are currently available have been derived from the plants directly or indirectly. Medicinal plants are an integral or essential part of human society for the treatment of diseases from the beginning of civilization [14–16] and provide continued important therapeutic agents, in both the modern and traditional medicine [15, 17]. In recent times, the utilization of medicinal plants belonging to different families is increasing because they are safe, effective, widely and

readily available and inexpensive as compared to modern medicine [18–20]. At least 89% of Africans depend on herbal medicine for their healthcare [15, 21]. Because of the linked side effects of the modern medicine, the traditional medicines are gaining importance and are now being evaluated to discover the scientific basis of their healing activities [15, 22] in terms of their antioxidant, antimutagenic and anticarcinogenic effects [23–25] and against a number of other diseases, such as antibacterial effect, since the plant products show fewer side effects and also may provide a way to the development of better new oral drugs [26, 27]. Nowadays, about 88% of the global populations turn to plant derived medicines as their first line of defence for their health maintenance and combating diseases [16, 19, 20, 26]. Plants possess a diverse variety of secondary metabolites, e.g. terpenoids, flavonoids, alkaloids and tannins that have been investigated in vitro to exhibit medicinal properties. About 120 secondary plant metabolites are used as drugs globally, 15% of all angiosperms have been evaluated chemically and of that 74% of pharmacologically active herbal components have been discovered [28]. Pharmacological studies have recognised the significance of medicinal plants as potential source of bioactive compounds [16, 19, 20, 26]. Extracts of some plants have been assessed in accordance with modern system of medicine and found to alter favourably the activities of several regulatory enzymes [29]. Medicinal plants are known to exhibit their antioxidative effects by scavenging the reactive oxygen species (ROS) and modulating the antioxidant defence mechanisms [30, 31]. The world market for alternative medicine is being increased to approximately USD 10.895 billion according to a recent report by the World Health Organization (WHO) [32]. The global market for plant derived medicine alone has reached $5 trillion and is increasing at a rate of about 11% per annum. This rapid growth in the market of the herbal/alternative medicines indicates its acceptance and popularity worldwide [32].

Cinnamon (*Cinnamomum cassia*, Family *Lauraceae*) bark is commonly used in Asian countries as a spice for most foods. In Eastern and Western folk medicine, it is used for treating abdominal and chest pains, chronic diarrhoea, hypertension, kidney disorders and rheumatism. It has strong antioxidant, analgesic, antiulcer, hypocholesterolaemic properties as well as antibacterial and anti-candidial activities and regulates the lipogenesis [33–36].

To our knowledge, no previous data are available on the cinnamon (*C. cassia*) extract to combat metal-induced toxicity in different organisms. Therefore, this study was designed to investigate the amelioration of the oxidative stress and hepatotoxicity induced by TiO_2 or its nanoparticles by cinnamon extract in male Sprague-Dawley rats.

Materials and Methods

The study was carried out at the Research Laboratory of the Department of Zoology, Government College University, Faisalabad. The details of materials and methods are discussed below.

Animals

Thirty-five healthy postweaning male Sprague-Dawley rats were procured from the animal house of Government College University, Faisalabad, housed in groups in ventilated cages under standard lighting conditions and natural day/night cycle after approval from the local ethical committee of the Government College University, Faisalabad. They were given a free access to water and food. Humidity and temperature (25 °C ± 2 °C) was controlled.

After a period of acclimatization for 7 days, the animals of similar mean initial body weights were randomly divided into groups, each having five animals (Table 1). The treatment groups receiving the higher tested sublethal dose (150 mg/kg bodyweight) of TiO_2 or TiO_2 nanoparticles were orally administered with the extract of cinnamon to analyse its healing activities.

A control group was fed by usual water and food, while the other group was treated with normal saline subcutaneously for the equivalency of the shock due to the injection. The animals receiving 150 mg/kg bodyweight TiO_2 NPs along with cinnamon extract at the dose of 50 or 100 or 150 mg/kg body weight orally were designated as group 2, group 3 and group 4, respectively, whereas the animals receiving TiO_2 bulk salt at the dose of 150 mg/kg body weight along with cinnamon extract at the dose of 50 or 100 or 150 mg/kg body weight orally were designated as group 5, group 6 and group 7, respectively. The treatment continued on alternate days for a period of 28 days. The dose of TiO_2 NPs and their bulk counterpart was selected on the basis of our previously determined sublethal dose at the rate of 150 mg/kg bodyweight of rat [11].

Sample Collection

Blood sample of all animals was collected at the start of the experiment and after 28 days of the treatment for the analysis of haematology, liver function tests, micronucleus assay and oxidative stress enzymes. At the end of the experimental period, animals were fastened overnight, anaesthetized the next day, by administering ketamine hydrochloride (30 mg/kg BW) and sacrificed. Blood samples were collected in heparinised tubes, and plasma was separated by centrifugation at 2000×*g* for 10 min. The livers were collected, weighed with the help of sartorius weighing balance and were separately immersed in fixative sera for further process of histology (by haematoxylin-eosin staining method).

TiO_2 Nanoparticles

The titanium dioxide nanoparticles were synthesized using sol-gel method from titanium isopropoxide [TTIP;

Table 1 Division of animals into different treatment groups

Groups	Treatments
Group 1	Control (no treatment)
Group 1(a)	Placebo (normal saline subcutaneously)
Group 2	TiO_2 NPs 150 mg/kg BW subcutaneously + CE 50 mg/kg BW orally
Group 3	TiO_2 NPs 150 mg/kg BW subcutaneously + CE 100 mg/kg BW orally
Group 4	TiO_2 NPs 150 mg/kg BW subcutaneously + CE 150 mg/kg BW orally
Group 5	TiO_2 BS 150 mg/kg BW subcutaneously + CE 50 mg/kg BW orally
Group 6	TiO_2 BS 150 mg/kg BW subcutaneously + CE 100 mg/kg BW orally
Group 7	TiO_2 BS 150 mg/kg BW subcutaneously + CE 150 mg/kg BW orally

$Ti(OC_3H_7)_4$] and characterized using XRD, SEM and EDX. The details of the synthesis and characterization of the nanoparticles and the preparation of solutions have been described in our previously published article [11].

Preparation of Cinnamon Extracts

The cinnamon (*Cinnamomum cassia*) bark of the medicinal quality was purchased from a local homeo store, and its botanical position was confirmed from the botany department of Government College University, Faisalabad. The cinnamon bark extract was prepared by following the method of plant extraction described by Khan et al. [37].

Body Weight

The body weights of the control group and all the experimental groups were observed and recorded weekly to note weekly changes in body weights.

Haematological Analysis

Blood samples were analysed using a haematology autoanalyser for the analysis of red blood cells (RBC) and white blood cells (WBC) counts, haemoglobin (Hb), platelets (PLT), neutrophils, monocytes, eosinophils, total leukocyte count (TLC), erythrocyte sedimentation rate (ESR), total protein, packed cell volume (PCV), erythrocyte indices like mean corpuscular value (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Liver Function Tests

The liver function tests such as ALT (alanine aminotransaminase), AST (aspartate aminotransferase) and ALP (alkaline phosphatase) were performed to analyse the effects of TiO_2 or TiO_2 nanoparticles as well as the cinnamon extract on the liver using standard methods [11].

Micronucleus Assay

All the blood samples were coded and processed immediately after collection. Slides were prepared for the micronucleus test [11].

Analysis of Oxidative Stress

The activities of biomarkers of oxidative stress, i.e. catalase activity, superoxide dismutase activity, glutathione-S-transferase activity and lipid peroxidation, were measured to determine the oxidative stress [11].

Histological Examination

The animals were euthanized and fresh portions were cut rapidly from lateral lobes of the liver of each rat. The samples were prepared for light microscopic analysis for liver histology following the haematoxylin and eosin staining technique [11].

Statistical Analysis

Data were statistically analysed with the help of Minitab17 software using ANOVA in general liner model to determine the treatment effects on different parameters. The analysis compared the effect of the abovementioned treatments on body weight, liver function tests, lipid peroxidation, oxidative stress and micronucleus assay parameters at $P < 0.05$. Tukey's test was used to compare treatments means at $P < 0.05$. Histology was assessed by recording visual observations of the photographic illustrations of liver tissues.

Results

The ameliorating activities of *C. cassia* were assessed by analysing the haematology, micronucleus assay and the liver histology of the treated rats.

Characterization of the Synthesized TiO_2 Nanoparticles

XRD analysis of the nanoparticles showed the average crystallite size of the said nanoparticles as 32.12 nm. The SEM

microphotograph for the synthesized TiO_2 nanoparticles showed the particles in the range of 30–80 nm [11].

Role of *C. cassia* as Antioxidant

The ameliorating activities of *C. cassia* against toxic effects caused by the sublethal doses of the subcutaneously administered TiO_2 nanoparticles as well as their bulk counterparts were demonstrated by analysing the haematology, liver function tests, micronucleus assay, oxidative stress enzymes as well as the histology of the treated rat.

Physiological Changes

Subcutaneous injection of 150 mg/kg TiO_2 or TiO_2 nanoparticles along with oral administration of 50 or 100 or 150 mg/kg *C. cassia* extract caused physiological changes. The rats exposed with 150 mg/kg of TiO_2 or TiO_2 NPs along with lower dose of extract (50 mg/kg) showed reduced weights and mortality of two animals during the last week of the experiment. The health status and behaviour of the animals with oral administration of 100 or 150 mg/kg of *C. cassia* extract along with 150 mg/kg TiO_2 or TiO_2 nanoparticles showed normal behaviour and their weights were increased. Table 2 shows significant increase in body weight in some groups and decreased weight in the other ones.

Haematological Analysis

Oral administration of *C. cassia* extract showed some ameliorative effects on the haematological alterations in rats caused by subcutaneous injection of TiO_2 or TiO_2 nanoparticles. At the start of the experiment, no significant changes were observed among all groups before administration of TiO_2 or TiO_2 NPs. Table 3 shows mean ± SD values of the haematological parameters among all groups at the start of the experiment.

Oral administration of *C. cassia* extract exhibited some ameliorative effects in rats after 28 days of exposure to TiO_2 or TiO_2 NPs as shown in Table 4.

Liver Function Tests

Liver function tests such as ALT, AST and ALP were performed to study liver damage of control and treated groups. After 28 days of exposure, the levels of ALT, AST and ALP were increased significantly at 150 mg/kg of TiO_2 nanoparticles and bulk salt TiO_2 along with low dose (50 mg/kg) of *C. cassia* extract, while at the highest dose (150 mg/kg) of *C. cassia* extract, the level of these enzymes gradually decreased. The oral administration of *C. cassia* significantly reduced the concentrations of ALT, AST and ALP. Figure 1 shows TiO_2 or TiO_2 nanoparticles-induced detectable increase

Table 2 Weekly body weight (g) of control groups and treated groups exposed to TiO_2 nanoparticles along with oral administration of *C. cassia* extract for 28 days

Ti me interval	Groups							
	Group 1 (control)	Group 1(a) (placebo)	Group 2 (NPs + 50 mg/kg CE)	Group 3 (NPs + 100 mg/kg CE)	Group 4 (NPs + 150 mg/kg CE)	Group 5 (BS + 50 mg/kg CE)	Group 6 (BS + 100 mg/kg CE)	Group 7 (BS + 150 mg/kg CE)
Initial weight	56.0 ± 0.6	54.6 ± 0.9	57.3 ± 0.9	54.0 ± 0.6	47.0 ± 0.6	67.0 ± 0.6	53.0 ± 0.6	45.0 ± 1.2
1st week	56.0 ± 0.6	58.0 ± 1.2	51.0 ± 3.5	53.3 ± 1.2	45.6 ± 0.7	68.3 ± 0.9	54.6 ± 1.2	55.3 ± 0.3
2nd week	64.3 ± 2.6	62.2 ± 1.3	43.6 ± 1.2	54.0 ± 1.2	50.3 ± 1.8	77.0 ± 1.2	60.3 ± 0.9	60.3 ± 1.8
3rd week	72.3 ± 1.8	66.2 ± 1.0	43.0 ± 0.6	58.3 ± 2.2	58.0 ± 2.1	83.6 ± 1.2	64.0 ± 0.6	65.3 ± 0.9
4th week	78.0 ± 1.2	70.3 ± 1.5	42.1 ± 1.2	63.0 ± 0.6	60.3 ± 0.9	87.6 ± 1.5	67.0 ± 0.6	73.3 ± 3.8

Values as mean ± SD

Table 3 Mean ± SD of haematological parameters among the control and the treated groups at the start of the experiment

Parameters	Units	Group 1 (control)	Group 1(a) (placebo)	Group 2 (NPs + 50 mg/kg CE)	Group 3 (NPs + 100 mg/kg CE)	Group 4 (NPs + 150 mg/kg CE)	Group 5 (BS + 50 mg/kg CE)	Group 6 (BS + 100 mg/kg CE)	Group 7 (BS + 150 mg/kg CE)
MCV	(fl)	42.4 ± 0.1	42.3 ± 0.3	42.4 ± 0.1	42.3 ± 0.1	42.4 ± 0.1	42.3 ± 0.1	42.4 ± 0.1	42.3 ± 0.1
MCH	(pg)	13.5 ± 0.3	13.4 ± 0.2	13.6 ± 0.4	13.2 ± 0.2	13.4 ± 0.2	13.7 ± 0.2	13.5 ± 0.2	13.5 ± 0.4
MCHC	(g/dl)	17.4 ± 0.2	17.3 ± 0.1	17.5 ± 0.2	17.4 ± 0.2	17.7 ± 0.2	17.4 ± 0.2	17.7 ± 0.2	17.3 ± 0.2
HCT	%	21.5 ± 0.1	21.6 ± 0.2	21.5 ± 0.1	21.4 ± 0.1	21.5 ± 0.1	21.4 ± 0.1	21.4 ± 0.1	21.4 ± 0.1
Hb	(g/dl)	12.3 ± 0.2	12.2 ± 0.2	12.3 ± 0.2	12.3 ± 0.2	12.4 ± 0.2	12.3 ± 0.2	12.3 ± 0.2	12.4 ± 0.2
WBC	$\times 10^3/\mu L$	6.5 ± 0.1	6.4 ± 0.2	6.5 ± 0.1	6.4 ± 0.2	6.4 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
RBC	$\times 10^6$/Ml	6.5 ± 0.02	6.6 ± 0.1	6.4 ± 0.02	6.4 ± 0.1	6.4 ± 0.02	6.5 ± 0.02	6.4 ± 0.01	6.4 ± 0.01
LYM	%	42.3 ± 0.1	42.1 ± 0.3	42.3 ± 0.2	42.3 ± 0.02	42.3 ± 0.1	42.2 ± 0.1	42.3 ± 0.2	42.3 ± 0.2
PLT	$\times 10^3/\mu L$	178.2 ± 0.1	178.3 ± 0.02	178.2 ± 0.1	178.2 ± 0.2	178.2 ± 0.1	178.2 ± 0.1	178.2 ± 0.1	178.2 ± 0.1

Table 4 Mean ± SD of haematological parameters among the control and the treated groups at the end of the experiment

Parameters	Units	Group 1 (control)	Group 1(a) (placebo)	Group 2 (NPs + 50 mg/kg CE)	Group 3 (NPs + 100 mg/kg CE)	Group 4 (NPs + 150 mg/kg CE)	Group 5 (BS + 50 mg/kg CE)	Group 6 (BS + 100 mg/kg CE)	Group 7 (BS + 150 mg/kg CE)
MCV	(fl)	44.3 ± 0.2^{H}	46.2 ± 0.1^{G}	50.2 ± 0.1^{F}	51.6 ± 0.2^{E}	54.2 ± 0.2^{C}	54.7 ± 0.2^{B}	53.2 ± 0.1^{D}	56.5 ± 0.39^{A}
MCH	(pg)	15.5 ± 0.2^{H}	16.2 ± 0.1^{G}	17.5 ± 0.2^{F}	18.3 ± 0.2^{E}	19.2 ± 0.1^{C}	19.6 ± 0.2^{B}	18.5 ± 0.395^{D}	20.6 ± 0.2^{A}
MCHC	(g/dl)	28.5 ± 0.2^{F}	27.5 ± 0.4^{G}	33.23 ± 0.2^{E}	35.5 ± 0.2^{C}	35.2 ± 0.1^{D}	35.5 ± 0.4^{C}	35.6 ± 0.2^{B}	36.3 ± 0.2^{A}
HCT	%	30.2 ± 0.1^{G}	28.5 ± 0.2^{H}	33.47 ± 0.3^{E}	35.3 ± 0.2^{D}	36.6 ± 0.2^{B}	35.7 ± 0.2^{C}	37.2 ± 0.1^{A}	33.6 ± 0.2^{F}
Hb	(g/dl)	11.3 ± 0.2^{F}	11.6 ± 0.4^{E}	12.3 ± 0.2^{C}	12.5 ± 0.2^{B}	12.5 ± 0.4^{B}	12.5 ± 0.3^{B}	13.4 ± 0.2^{A}	12.2 ± 0.2^{D}
WBC	$\times 10^3/\mu L$	6.6 ± 0.2^{H}	8.2 ± 0.1^{F}	7.5 ± 0.4^{G}	8.5 ± 0.4^{E}	22.7 ± 0.2^{A}	20.5 ± 0.2^{B}	19.5 ± 0.39^{C}	9.3 ± 0.2^{D}
RBC	$\times 10^6$/Ml	6.8 ± 0.02^{B}	6.6 ± 0.02^{C}	5.34 ± 0.02^{F}	6.8 ± 0.02^{B}	6.8 ± 0.02^{B}	6.2 ± 0.6^{D}	6.98 ± 0.01^{A}	5.9 ± 0.02^{E}
LYM	%	38.2 ± 0.1^{E}	37.1 ± 0.1^{F}	31.2 ± 0.1^{H}	35.2 ± 0.1^{G}	66.5 ± 0.2^{D}	72.2 ± 0.2^{B}	68.5 ± 0.2^{C}	96.5 ± 0.3^{A}
PLT	$\times 10^3/\mu L$	177.0 ± 0.1^{G}	170.0 ± 0.1^{H}	1902.0 ± 0.1^{B}	1920.0 ± 0.1^{A}	1698.0 ± 0.1^{D}	1592.0 ± 2^{E}	1378.0 ± 2^{F}	1817.0 ± 2^{C}

Means that do not share a letter in the same row are significantly different

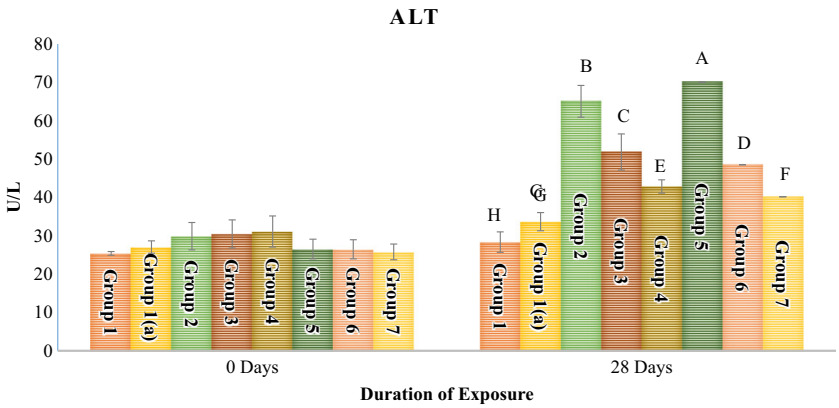

Fig. 1 Mean ± SD of ALT (U/L) among the control and the treated groups with TiO_2 or TiO_2 NPs along with *C. cassia* extracts

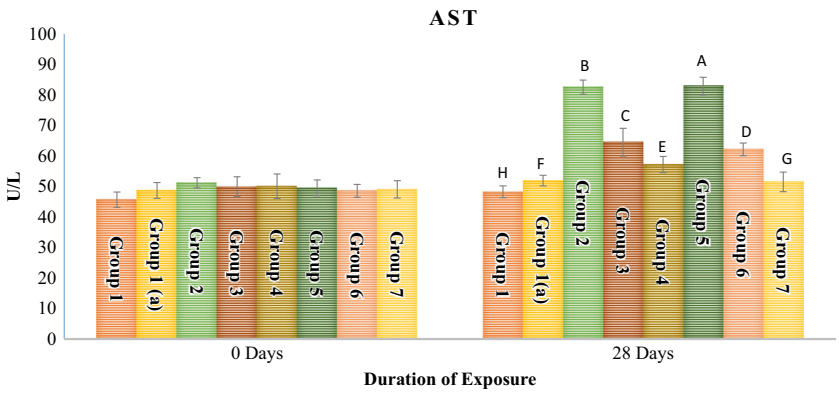

Fig. 2 Mean ± SD of AST (U/L) among the control and the treated groups with TiO_2 or TiO_2 NPs along with *C. cassia* extracts

in ALT levels in rat's blood with oral administration of *C. cassia* extract at low dose but the higher dose of *C. cassia* extract reduced the ALT levels in blood. Figure 2 also indicates similar effects on the levels of AST in rat groups treated with TiO_2 or TiO_2 nanoparticles as well as with oral administration of *C. cassia* extract. The low doses of the *C. cassia* extract (50 mg/kg body weight of rats) were least effective in ameliorating the alterations in the concentrations of the AST induced due to the subcutaneously injected TiO_2 or TiO_2 nanoparticles, while the higher dose (150 mg/kg body weight of rats) showed significant ameliorative effects on the blood levels of AST. It is also clear from the Fig. 3 that the *C. cassia* extract showed decreasing effects on the blood levels of ALP in groups treated with high doses (150 mg/kg body weight of rat) of *C. cassia* extract, whereas the low doses of *C. cassia* extract showed no obvious control on the TiO_2 or TiO_2 nanoparticles-induced alterations in the ALP concentrations in blood.

Micronucleus Assay

Micronucleus assay was performed to study micronucleus (MN) frequency of control and treated groups. After 28 days of exposure, the micronuclei frequency increased significantly

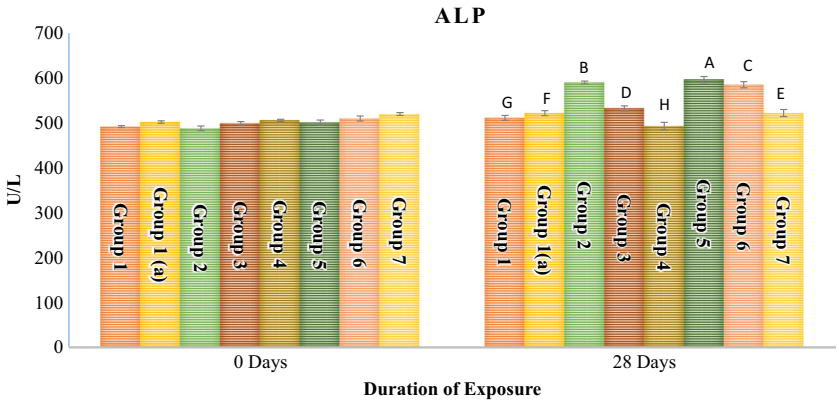

Fig. 3 Mean ± SD of ALP (U/L) among the control and the treated groups with TiO_2 or TiO_2 NPs along with *C. cassia* extracts

Table 5 Number of micronuclei after administration of different concentrations of *C. cassia* extract with TiO_2 or TiO_2 nanoparticles for 28 days

Groups	Exposure duration	
	0 days	28 days
Group 1	0.21 ± 0.02	0.30 ± 0.57^{H}
Group 1(a)	0.23 ± 0.02	0.41 ± 0.21^{G}
Group 2	0.23 ± 0.023	2.41 ± 0.01^{A}
Group 3	0.29 ± 0.05	2.12 ± 1.01^{C}
Group 4	0.13 ± 0.21	1.92 ± 0.61^{E}
Group 5	0.21 ± 0.02	2.20 ± 0.11^{B}
Group 6	0.23 ± 0.02	2.11 ± 0.13^{D}
Group 7	0.23 ± 0.02	1.80 ± 0.09^{F}

Values as mean ± SD for five replicates

at 150 mg/kg of TiO_2 nanoparticles or bulk salt of TiO_2 with low dose of 50 mg/kg of *C. cassia* extract, while at the highest dose (150 mg/kg) of *C. cassia*, the micronuclei frequency was gradually decreased. Table 5 shows TiO_2 or TiO_2 nanoparticles-induced detectable damage in rat's blood with oral administration of *C. cassia* extracts at low dose but the higher dose reduced the DNA damage due to the antioxidant properties of *C. cassia.*

Histology

After the oral treatment of *C. cassia* extract to Sprague-Dawley rats along with the subcutaneous injections of TiO_2 or TiO_2 nanoparticles for 28 days, the livers of the test animals were evaluated for the histological studies.

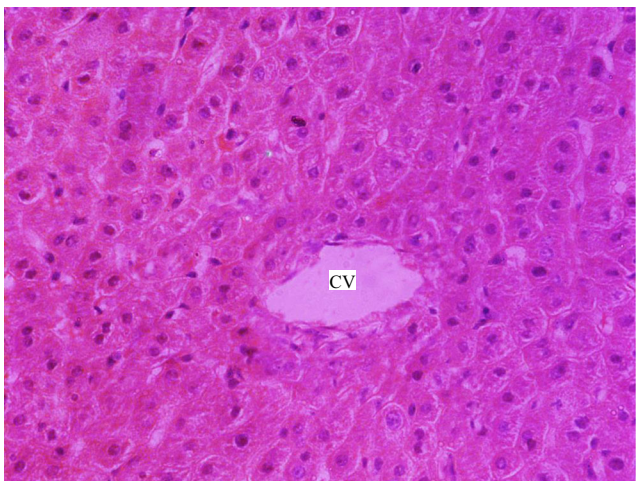

Fig. 4 Photomicrograph (×400) of haematoxylin-eosin stained section of the control liver (group 1) section showing normal histology with the normal cellular arrangement of hepatic lobule with centeral vein (*CV*) nearly at the center

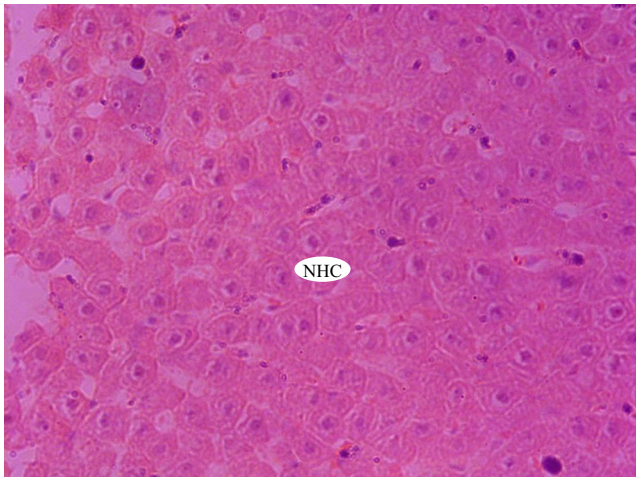

Fig. 5 Photomicrograph (×400) of haematoxylin-eosin stained section of group 1(a) (placebo group treated with only normal saline subcutaneously) showing normal histology with the normal architecture of hepatic lobule and normal hepatocytes (*NHC*)

Results showed that the extract had ameliorative effects on the histological damages caused by the TiO_2 bulk salt or the TiO_2 nanoparticles. The histological results are shown in Fig. 4 for group 1 (the control group receiving no treatments) and Fig. 5 for group 1(a) (the placebo group treated with only normal saline subcutaneously). Figures 6, 7, 8, 9, 10, and 11 show the liver histology of rats treated with subcutaneous injections of sublethal dose of TiO_2 or TiO_2 nanoparticles and various concentrations of *C. cassia* extract.

Oxidative Stress Indicators

After the 28 days of treatment with TiO_2 or TiO_2 NPs, the level of CAT was found decreasing in the liver and blood as

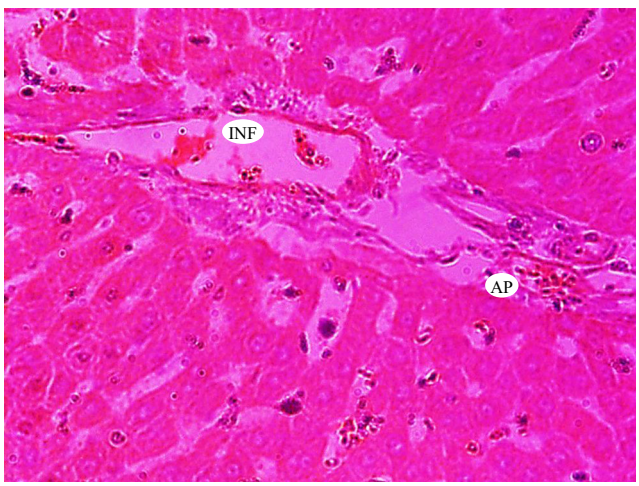

Fig. 6 Photomicrograph (×400) showing histological alterations of the liver section of group 2 treated orally with 50 mg/kg BW of cinnamon extract along with the subcutaneous injection of 150 mg/kg BW TiO_2 nanoparticles showing destructed blood vessels, infiltration (*INF*) of neutrophils and apoptosis (*AP*)

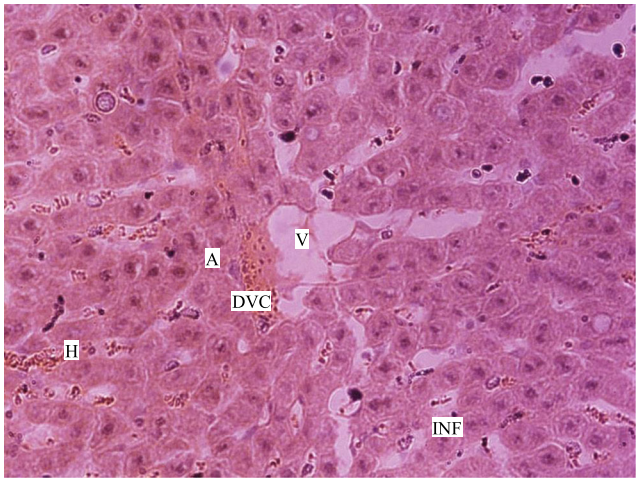

Fig. 7 Photomicrograph (×400) showing histological alterations of the liver section of group 3 treated orally with 100 mg/kg BW of cinnamon extract along with the subcutaneous injection of 150 mg/kg BW of TiO_2 nanoparticles showing damaged central wein (*DCV*), vaculation (*V*), apoptosis (*AP*), haemorrhage (*H*) and infiltration (*INF*)

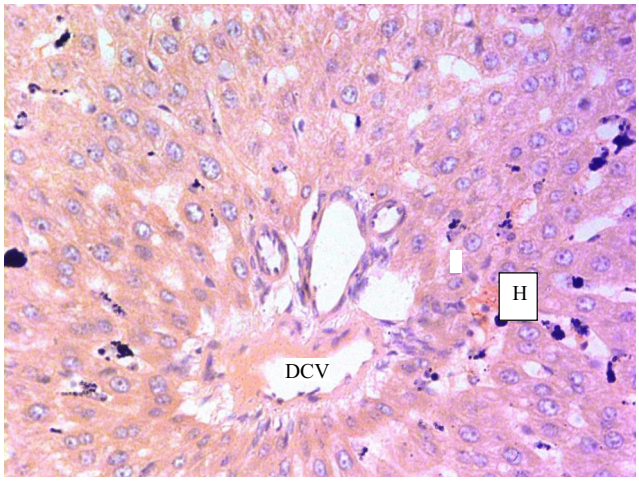

Fig. 9 Photomicrograph (×400) showing histological alterations of the liver section of group 5 treated orally with 50 mg/kg BW of cinnamon extract along with the subcutaneous injection of 150 mg/kg BW of TiO_2 bulk salt showing dilated congested vein (*DCV*) and haemorrhage (*h*)

compared to the control group (Fig. 12). However, the use of *C. cassia* increased the activity of CAT significantly ($P < 0.05$) as compared to the treated group of TiO_2 or TiO_2 NPs (Fig. 13).

TiO_2 or TiO_2 NPs significantly increased the activity of SOD after 28 days of treatment. However, the use of *C. cassia* ameliorated the oxidative stress and reduced the level of SOD in both the liver and blood (Fig. 14).

The use of TiO_2 or TiO_2 NPs significantly increased the level of lipid peroxidation as compared to the control group. Oral administration of *C. cassia* significantly reduced the level of lipid peroxidation (Fig. 15).

Discussion

Medicinal plants are being screened for the free radical scavenging properties based on the reports about their safety, efficacy and cost effectiveness [38–40] over the synthetic antioxidants that have been reported with side effects upon their consumption. As a result, a number of medicinal plants reported to have antioxidant properties are being recommended for pharmaceutical industries and traditional medicine to control and treat different types of ailments [41]. In recent times, phytotherapy has gained popularity all over the world. Many plant parts possess phytochemical compounds which may be categorized into three

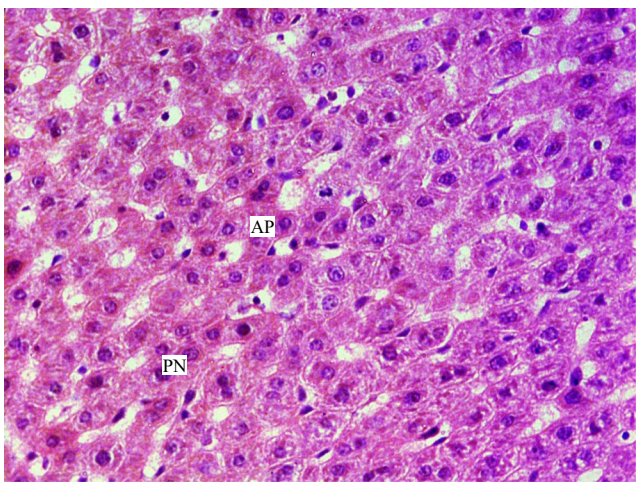

Fig. 8 Photomicrograph (×400) showing histological alterations of the liver section of group 4 treated orally with 150 mg/kg BW of cinnamon extract along with the subcutaneous injection of 150 mg/kg BW of TiO_2 nanoparticles showing minor histological alterations with minor apoptosis (*AP*) and pyknotic nuclei (*PN*)

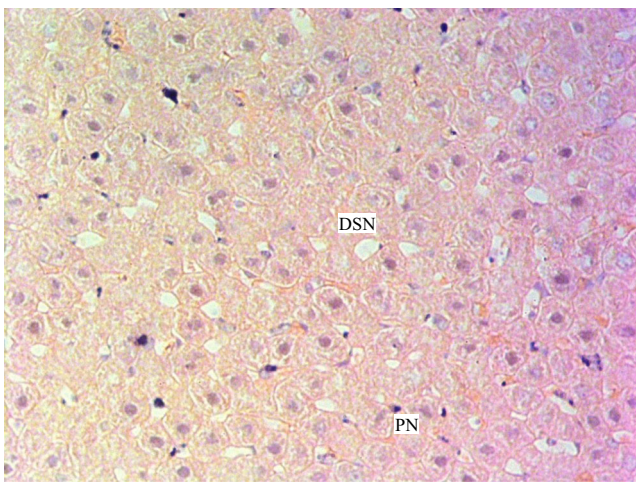

Fig. 10 Photomicrograph (×400) showing histological alterations of the liver section of group 6 treated orally with 100 mg/kg BW of cinnamon extract along with the subcutaneous injection of 150 mg/kg BW of TiO_2 bulk salt showing some pyknotic nuclei (*PN*) and dilated sinusoids (*DSN*).

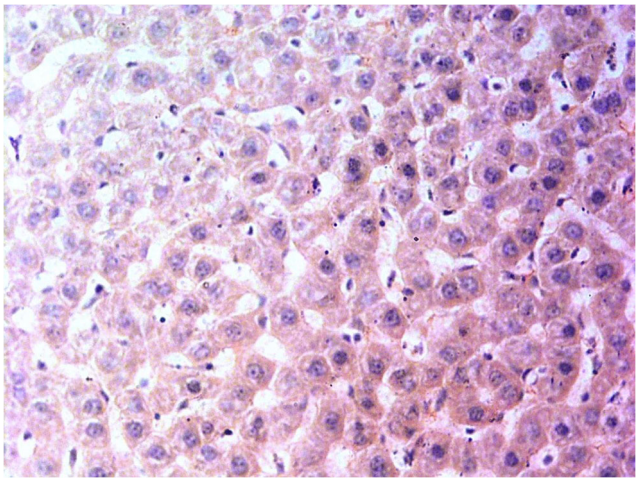

Fig. 11 Photomicrograph (×400) showing histological alterations of the liver section of group 7 treated orally with 150 mg/kg BW of cinnamon extract along with the subcutaneous injection of 150 mg/kg BW of TiO_2 bulk salt showing only minor apoptosis

major groups: the carotenoids, limonoids and flavonoids. Antioxidants interfere with oxidation reaction by reacting with free radicals, chelating catalytic metals, scavenging oxygen [42] and prevent lipid autoxidation [43, 44]. Researches have been performed worldwide for the potential of herbal medicine for their use in different fields of medicine. Use of herbal medicines exhibits positive effects in curing different diseases [45].

Cinnamon has been reported by a number of studies for having a reasonable amount of phenols and flavonoids. Polyphenolic and flavonoid compounds are important secondary metabolites in plants and are reported to be responsible for the variation in antioxidant activities in plants [46–48] and are capable of fighting against free radicals by inactivating lipid-free radicals or preventing decomposition of hydrogen peroxide into free radicals due to their redox properties, chelate metal ions, quenching singlet and triplet oxygen [49–52]. This may undoubtedly suggest that plants with high quantity of polyphenols and flavonoids are good antioxidant sources.

Proximate analyses indicate that cinnamon powder has been found to be rich in fibre and carbohydrates and also contains appreciable amounts of protein and some minerals [53, 54]. Cinnamon powder has also been found to have high total phenolic content and antioxidant activity [53, 55]. Cinnamon contains complex chemical profile that includes phenolic compounds. The phenolic compound is general

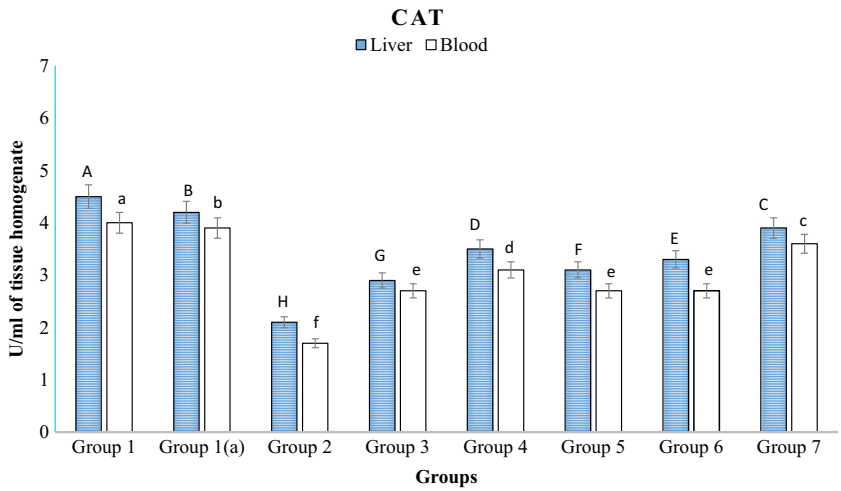

Fig. 12 Effect of various concentrations of orally administered *C. cassia* extract on the TiO_2 or TiO_2 nanoparticles-induced alterations in CAT activity (mean ± SD) after 28 days of exposure

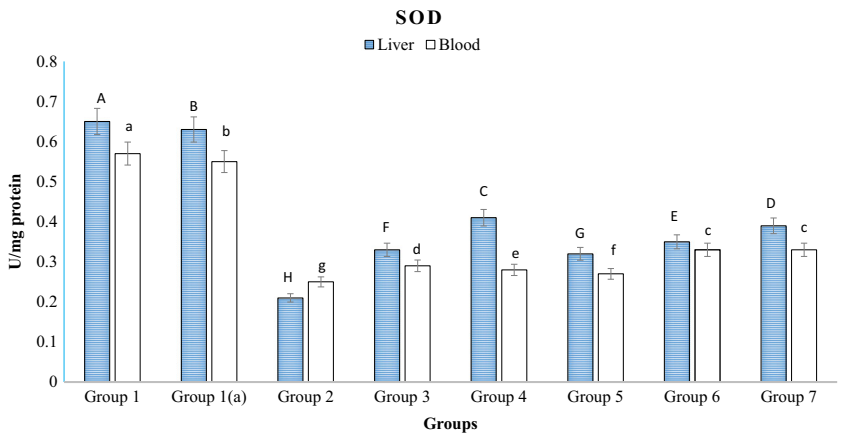

Fig. 13 Effect of various concentrations of orally administered *C. cassia* extract on the TiO_2 or TiO_2 nanoparticles-induced alterations in SOD activity (mean ± SD) after 28 days of exposure

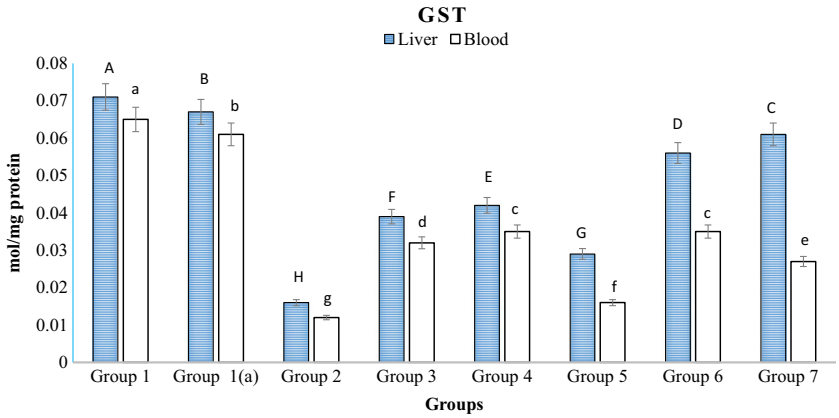

Fig. 14 Effect of various concentrations of orally administered *C. cassia* extract on the alterations of GST activity (mean ± SD) due to TiO_2 or TiO_2 NPs after 28 days of exposure

word and denotes to the compounds having at least one aromatic ring and one or extra attached hydroxyl group [56]. The phenolic compound possesses this capability due to the redox potential acting as reducing agent and reduces the singlet oxygen [57]. Flavonoid is considered one of most important phenolic compound with known properties and a significant role as free radical scavenging.

The current study investigated the ameliorative effects of *C. cassia* against the toxicity caused by the subcutaneously injected TiO_2 or TiO_2 nanoparticles in the body of albino rats. This study revealed the remedial or healing effects of *C. cassia* (cinnamon) in rats exposed to TiO_2 sublethal doses. This study showed that TiO_2 and TiO_2 NPs exposure for 28 days with oral administration of cinnamon extract at dose of 100 or 150 mg/kg was able to minimize the toxic effects of TiO_2 or TiO_2 NPs and induced healing effects in liver pathology and decreased the DNA damage and oxidative stress due to TiO_2 and TiO_2 NPs in albino rats. The findings of this study are in agreement with the results of many scientists who found that cinnamon has antioxidant properties [33–35, 45, 58–67].

In this study, nearly normal haematological parameters were observed when cinnamon extract was administered along with the TiO_2 or TiO_2 nanoparticles. A number of other researches are in support to these findings. Cinnamon, a natural product with a long history of safety, is rich in polyphenolic components that have been revealed to improve the action of insulin in vitro [68], in animal researches [69, 70] and to possess in vitro antioxidant activity [71]. In this study, results showed that the administration of cinnamon significantly improved SOD, CAT and GST and decreased lipid peroxidation in dose-dependent manner. Similar findings were observed in a research by Khaki et al. [45] where the administration 75 mg/kg *Cinnamon zeylanicum* caused significantly increase in serum SOD, CAT, GPX and decreasing MDA levels in rats. This results due to its flavonoids and related compounds. Researches confirmed that flavonoid especially phytoestrogens have the potential to affect steroid biosynthesis and metabolism through a number of pathways [45, 72]. Markedly, antioxidant activity of cinnamon was also shown in liver tissue of the Wistar albino rats by [Noori et al. [33], Moselhy and Ali [61]] and Eidi et al. [73]. Normal and healing effects of cinnamon extracts have been demonstrated in a number of researches. Pretreatment of male Wistar rats with cinnamon extract considerably preserved the structure of hepatic cells and markedly reduced the hepatotoxicity caused by carbon tetrachloride (CCl_4) [61, 73] and oral treatment of cinnamon bark extract at the dose of 250 and 500 mg/kg BW to diabetic rats revealed completely repaired histology of the

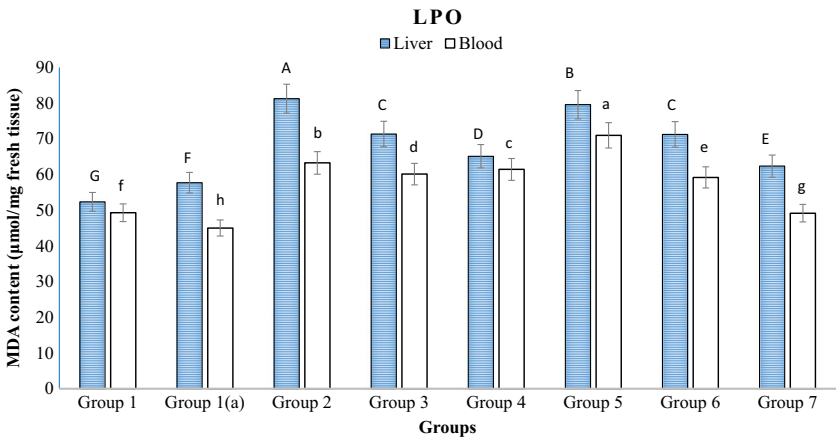

Fig. 15 Effect of various concentrations of orally administered *C. cassia* extract on the alterations of LPO (mean ± SD) due to TiO_2 or TiO_2 NPs after 28 days of exposure

testis of the diabetic rats [74]. A recent study showed the ability of cinnamon to cure the tubular injury of the kidney of female Wistar rats caused by gentamicin apart from having phenolic compounds and antioxidant properties [75]. Oral administration of cinnamon oil significantly restored liver function and normalized the histological, histochemical and biochemical anomalies in alloxan-treated diabetic rats, might be due to antioxidant properties [76].

In this study, cinnamon-treated rats also showed approximately normal values of some haematological parameters like RBC, Hb, HCT, MCH and MCHC supported by a number of studies. Ahmad et al. [77] reported recently no significant effects of 0.1 or 0.5 g/kg BW cinnamon extract on the behaviour, mortality, food consumption, water intake, weight gain, liver histology and haematological parameters in Sprague-Dawley rats. Cinnamon extract significantly protected the Hb, HCT, MCH and MCHC decrease in frog *Rana ridibunda* caused by CCl_4 [78]. Molla et al. [79] reported that 1 mL/l cinnamon extract in drinking water caused no change in haematological parameters in broilers. Similarly, 0.8% cinnamon increased the values of RBC and Hb concentrations [80]. However, the values WBC, LYM and PLT increased, that may be due to the provocation of the immune response. So, it is concluded that *Cinnamomum cassia* (cinnamon) showed remedial or healing effects in rats exposed to TiO_2 sublethal doses as this plant is known for its antioxidative properties.

Conclusion

Oral administration of *C. cassia* (cinnamon) extract at doses of 100 or 150 mg/kg reduced the toxic effects of exposure with subcutaneous injection of TiO_2 or TiO_2 NPs in rat and reduced the liver injuries that were evident from the histological studies as well as the liver function enzymes concentrations. Therefore, cinnamon extract added in the recovery of the antioxidant system as well as protective role in haematological and histological damages in the rat liver treated with TiO_2 or TiO_2 NPs.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in this study involving rats were in accordance with the research ethical standards of the Government College University, Faisalabad, Pakistan.

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