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ORIGINAL ARTICLE

Taurine and protocatechuic acid attenuate Vincristine sulphate-induced bone marrow, liver and intestinal injuries via anti-oxidative, anti-inflammatory and anti-apoptotic activities

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Abstract

Chemotherapy with Vincristine (Vcr) is often compromised by undesirable gastrointestinal, myeloid and hepatic effects. In this study, we evaluated and compared the efficacy of taurine (Tau) and/or protocatechuic acid (Pca) in alleviating Vcr-induced hepatotoxicity, enterotoxicity and myelotoxicity in rats. In two cycles of five daily injections each, rats were exposed to Vcr (0.1 mg/kg, i.p.) alone or in combination with orally administered Tau (50 mg/kg) and/or Pca (50 mg/kg). Blood was collected for haematology and measurement of liver enzymes and inflammatory cytokines. Genotoxicity assay was performed on bone marrow, while the liver and intestines were subjected to biochemical assays, histopathology and immunohistochemical staining. Administration of Vcr triggered bone marrow suppression (anaemia, leucopenia, thrombocytopenia and increased frequency of micronucleated polychromatic erythrocytes, MnPCEs), increased serum transaminases (ALT, AST) and alkaline phosphatase (ALP) and altered hepatic and intestinal morphology. However, supplementation with Tau and/or Pca alleviated most of the toxic effects of Vcr by reducing tissue levels of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and advanced oxidation protein products (AOPP), but stimulating glutathione S-transferase (GST) and glutathione peroxidase (GPx) activities. In addition, Tau and/or Pca enhanced anti-inflammatory (reduced serum TNFα) and anti-apoptotic mechanisms (reduced cytochrome c/Bax expression and increased Bcl-2 expression) in the ileum and liver. Overall, Tau or Pca protected the liver, ileum and bone marrow against Vcr-induced toxicities via antioxidant, anti-inflammatory and anti-apoptotic mechanisms. The data supports their individual use, rather than their combination, as adjuvant therapy in patients undergoing chemotherapeutic intervention.

Keywords Vincristine · Taurine · Protocatechuic acid · Liver · Bone marrow · Ileum · Oxidative stress · Apoptosis

Introduction

Vincristine (Vcr) is an antineoplastic drug belonging to the vinca alkaloids group and is commonly used in the treatment of human cancers such as leukemias, lymphomas and sarcomas (López-Gómez et al. 2018). In veterinary medicine, Vcr is often the drug of choice for the chemotherapy of canine transmissible venereal tumor (Setthawonsin et al. 2019). The anticancer action of Vcr is mediated by its ability to bind to β-tubulin in tubulin heterodimers resulting in disorganization of cellular microtubule cytoskeleton, inhibition of cell cycle progression in mitotic cells and apoptosis (Babu et al. 2015). Like most chemotherapeutic drugs, the efficacy of Vcr is limited by associated undesirable effects in various tissues, especially rapidly proliferating normal cells. Common side effects of Vcr include gastrointestinal effects (e.g. constipation, hypomotility, anorexia, abdominal cramps, weight loss, etc.), nervous effects (e.g. peripheral neuropathy, urinary retention, paraesthesias, muscle weakness and atrophy) and myelosuppression in bone marrow tissues (Conklin 2000; Peixoto unior et al. 2009; Dhyani et al. 2022). However, unlike most other chemotherapeutic drugs, Vcr is believed to exhibit relatively lesser bone marrow suppression (López-Gómez et al. 2018).

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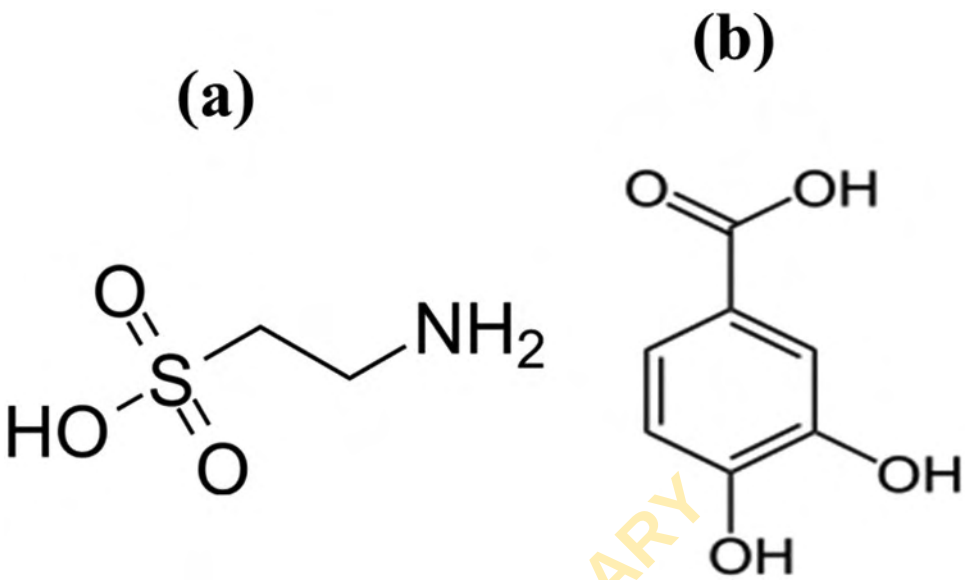

Fig. 1 Structures of (**a**) Taurine (2-aminoethanesulfonic acid) and (**b**) Protocatechuic acid (3, 4-dihydroxybenzoic acid)

Most chemotherapeutic drugs, including Vcr, are able to kill cancer cells by activating apoptotic pathways involving the release of cytochrome c from the mitochondria, while most side effects have also been associated with premature diversion of electrons from the electron transport chain to oxygen via NADH dehydrogenase, resulting in formation of reactive oxygen species (ROS), such as superoxide radicals (Conklin 2004). When present in excessive amounts, ROS generation tend to overwhelm cellular antioxidant mechanisms, causing oxidative stress. Under these conditions, ROS can interact with cellular macromolecules, including DNA, proteins and lipids to cause tissue damage and interference with vital cellular functions. For instance, damage to intestinal structure after Vcr administration has been observed in rats as part of its adverse gastrointestinal effects (López-Gómez et al. 2018). Therefore, it becomes highly imperative that research be carried out to elucidate the potential of single antioxidants or mixtures of antioxidants for mitigating side effects of anticancer drugs available for clinical use.

Taurine (Tau; 2-aminoethanesulfonic acid) (Fig. 1a), a sulphur-containing semi-essential amino acid often regarded as a functional nutrient, can be supplied from food intake or hepatic synthesis via a reaction between methionine and cysteine (Sarkar et al. 2017). Evidence suggests that Tau exhibits cytoprotection by promoting antioxidant actions and modulation of inflammatory processes (Sirdah 2015). Although Tau is not normally thought to be a classical ROS scavenger or an inducer of antioxidant enzymes, its antioxidant activities are believed to be mediated by its involvement in mitochondrial protein synthesis with enhancement of electron transport and prevention of excessive generation of superoxide radicals in the mitochondria (Jong et al. 2012). Previous studies indicate that Tau protects the small intestine against oxidative stress-mediated disruption of the tight-junction barrier induced by chemicals, ischaemia–reperfusion and lipopolysaccharides (Sukhotnik et al. 2016; Xiao et al. 2018; Wen et al. 2020).

Protocatechuic acid (Pca; 3, 4-dihydroxybenzoic acid) (Fig. 1b), is a naturally-occurring flavonoid with antioxidant and anti-inflammatory properties found in many vegetables, herbs, fruits and spices (Zhang et al. 2021). The antioxidant mechanisms of Pca include direct radical scavenging; stimulation of endogenous antioxidant enzyme activity, including glutathione peroxidase (GPx) and superoxide dismutase (SOD) as well as inhibition of ROS production from xanthine oxidase and NADPH oxidase (Krzysztoforska et al. 2019). Previous studies in mice have shown that Pca could protect the intestinal barrier against dysfunction induced by bile duct ligation (Ning et al. 2013), while other studies have reported protective effects of Pca against liver damage in rats via reduction of oxidative stress (Kakkar and Bais 2014).

Despite the abundance of Tau and Pca as naturally occurring compounds with desirable biological effects, there has been no research on their benefits during Vcr chemotherapy. The goal of this study was, therefore, to elucidate the possible protective roles of Tau or Pca or their combination in Vcr-induced toxicity involving the liver, bone marrow and intestines, as well as the underlying oxidative, inflammatory and/or apoptotic mechanisms in rats.

Materials and methods

Chemicals, reagents and kits

All the reagents and chemicals used in this study were of analytical grade. Vincristine sulphate (Vinlon^{TM}1) is a product of CELON Laboratories Pvt. Ltd (Telangana, India) and was purchased from a reputable pharmaceutical store in

Ibadan, Nigeria. Taurine, Protocatechuic acid, thiobarbituric acid (TBA), trichloroacetic acid (TCA), Xylenol orange, Tris Base, sodium hydroxide, reduced glutathione (GSH), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), 1, 2-dichloro-4-nitrobenzene (CDNB), and other major reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tumor Necrosis Factor alpha (TNFα) and Interleukin-1 beta (IL-1β) kits were purchased from Elabscience® Biotechnology Co. Ltd (Wuhan, Hubei, China). Spectrophotometric enzyme assay kits for Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were purchased from Randox® Laboratories Ltd. (Randox® Laboratories Ltd, Ardmore, United Kingdom). Anti-Cytochrome c, anti-bax and anti-Bcl-2 antibodies were obtained from Dako North America Inc. (Real Carpinteria, CA, USA).

Animals

Male Wistar rats weighing 100–130 g were purchased from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The rats were housed in plastic cages in a well-ventilated animal house with constant temperature (22 ± 2 ºC) and a photoperiod cycle of 12 h light and 12 h dark. The animals were allowed standard rat chow and water ad libitum. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The handling and treatments of all animals was in strict adherence to guidelines locally approved by the Animal Care and Use Committee of our institution as well as NIH guidelines outlined in the "Guide for the Care and Use of Laboratory Animals" (Public Health Service 1996).

Experimental design and drug administration

Fifty male Wistar rats were divided into five groups of 10 animals each and were treated with Vcr, Tau and Pca as follows:

Group A (Control): Apparently normal rats receiving normal saline (2 ml/kg) only
Group B (Vcr): Rats injected with Vcr only at 0.1 mg/kg intraperitoneally in two cycles comprising daily injections for five consecutive days in each cycle. An interval of two days was allowed between the two cycles.
Group C (Vcr+Tau): Rats injected with Vcr and concurrently with Tau (50 mg/kg) orally for 12 consecutive days
Group D (Vcr+Pca): Rats injected with Vcr and concurrently with Pca (50 mg/kg) orally for 12 consecutive days
Group E (Vcr+Tau+Pca): Rats injected with Vcr and concurrently with Tau (50 mg/kg) and Pca (50 mg/kg) orally for 12 consecutive days

The doses, routes of administration and duration of treatment with the different compounds were chosen based on previous studies (Vcr: (López-Gómez et al. 2018); Pca: (Adedara et al. 2019; Owumi et al. 2019); Tau: (ElBanna et al. 2023)). Specifically, 0.1 mg/kg of Vcr was reported by Lopez-Gomez et al. to produce villi shortening and large inflammatory nodules in the ileum and colon, which were still evident 2 weeks after treatment. The 50 mg/kg doses of Tau and Pca were the highest effective doses in the previous experiments where they were employed. For administration, Vincristine sulphate was dissolved in physiological saline (0.9% NaCl) and was administered by intraperitoneal injection at a dose of 0.1 mg/kg daily. The day prior to the first administration was taken as day 0 when the initial weights of the rats were measured with subsequent measurements every three days and upon termination of the experiments on day 13. In groups taking drug combinations, the administration of Vcr was separated from that of other compounds by at least an hour to prevent drug interactions that may affect the activity of each drug.

Sample collection and preparation

On day 13, approximately 24 h after the last administration, blood samples were collected from the retro-orbital plexus of each rat under slight anaesthesia into heparinized and non-heparinized tubes for determination of haematological and serum biochemical parameters. The blood collected in non-heparinized tubes was allowed to clot for about an hour and was later centrifuged at 3,000 rpm for 10 min at room temperature and the clear supernatant was collected as serum. The rats were subsequently euthanized by cervical dislocation and the bone marrow, liver, ileum and colon were dissected out. The tissues were rinsed in ice-cold normal saline (0.9% NaCl), blotted on dry filter paper and weighed. One portion of the tissues was processed for biochemical analysis and a smaller portion was reserved for histopathological examination. Tissues for biochemical assays were homogenized in phosphate buffer (pH 7.4). The homogenates was thereafter centrifuged in a refrigerated centrifuge (4 ºC) for 10 min to obtain the post-mitochondrial fraction.

Haematological evaluation and assay of serum enzymes

Whole blood collected in heparinized tubes was used for determination of haematological parameters including packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), platelet count, neutrophils and lymphocytes using an Auto Haematology analyzer (SYSMEX Automated Haematology Analyser, United Kingdom). The collected serum was used for determination of the activities of Alanine

aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP).

Genotoxicity assay

Genotoxicity induction by Vcr was determined in the bone marrow cells harvested from the femur bones of the rats using the micronucleus assay as described by Heddle and Salamone (Heddle and Salamone 1981). Bone marrow was flushed from both femur bones and smeared onto plain glass slides using fetal calf serum (FCS). The slides were air-dried for up to 24 h after which they were fixed by dipping in methanol for 5 min, dried and then stained with May-Gruenwald and Giemsa stains. The slides were rinsed in phosphate buffer and distilled water, air-dried and cover slips were applied. Thereafter, the slides were viewed with a light microscope and the frequency of micro-nucleated polychromatic erythrocytes (MnPCEs) per 1000 PCEs was scored by an observer who was blinded to the experimental design.

Biochemical analysis

Hydrogen peroxide (H_2O_2) concentration in hepatic and intestinal tissues was measured spectrophotometrically at a wavelength of 560 nm according to the method of Wolff (Wolff 1994). Lipid peroxidation in the tissues was quantified as the content of malondialdehyde (MDA), with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ using methods described by (Varshney and Kale 1990). Advanced oxidation protein products (AOPP) was measured as index of protein oxidation in the tissues according to the methods described by (Kayali et al. 2006). The concentration of reduced glutathione (GSH) in the samples was quantified using the methods of Jollow et al. (Jollow et al. 1974). The activity of Glutathione S-transferase (GST) was determined according to the method of (Habig et al. 1974) which measures the ability of the enzyme to catalyze the conjugation of GSH with 1-chloro-2, 4-dinitrobenzene (CDNB) over a time period of about 3 min with absorbance read at 340 nm in 30-s intervals. Glutathione peroxidase (GPx) activity was determined by using the method of (Rotruck et al. 1973).

Measurement of inflammatory cytokines

The serum levels of Tumor necrosis factor alpha (TNFα) and Interleukin 1 beta (IL-1β) were measured with the aid of commercial enzyme-linked immunosorbent assay (ELISA) kits (Elabscience Biotechnology Inc., Houston, Texas, USA) (TNFα Catalog number: E-EL-R0019; IL-1β Catalog number: E-EL-R0019).

Histopathology

Small portions of freshly isolated liver, ileum and colon were immediately transferred into 10% buffered formalin and processed for histopathological examination according to methods described by (Drury et al. 1976). Briefly, the tissues were first dehydrated in ethanol, embedded in paraffin and sections of about 4–5 µm were made with a rotary microtome. The sections were fixed on plain glass slides and were prepared for staining with haematoxylin and eosin (H&E). The stained slides were, thereafter, examined under a light microscope for general observation of lesions.

Immunohistochemistry

Ileum and liver sections were prepared from formalin-fixed, paraffin-embedded tissues and these were subjected to immunohistochemistry protocols according to the methods described by (Todorich et al. 2011). The sections were first deparaffinized and rehydrated using xylene and graded concentrations of ethanol. Following antigen retrieval in a citrate/EDTA buffer and blocking with 10% fetal bovine serum, the tissue sections were incubated with primary anti-cytochrome c, anti-bax and anti-Bcl-2 antibodies (Dako North America Inc. Real Carpinteria, CA, USA). Thereafter, appropriately diluted labelled Polymer-HRP secondary antibody was also applied to the slides and also incubated. Subsequently, freshly prepared diaminobenzidine (DAB) color solution was added to the sections and they were then counterstained with haematoxylin. Regions of protein expression were observed as brownish areas using a light microscope and photomicrographs were generated with a digital camera. The percentage (%) area of protein expression was calculated from 10–15 miscroscopic fields using ImageJ (Fiji) software (version 1.53c, National Institutes of Health, USA) to generate semi-quantitative values.

Statistical analysis

Data obtained from different measurements were analyzed using GraphPad Prism software (version 7.00) and were expressed as mean ± standard deviation. The differences among means was determined by One-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons, while P values less than 0.05 were considered statistically significant.

Results

Effects of taurine and protocatechuic acid on body weight, liver and *colon* weights in Vincristine-treated rats

The final body weights recorded in rats from all experimental groups were significantly higher than the initial weights at the beginning of the experiment (Table 1). At the commencement

of the experiments, the average weights of all experimental groups were not significantly different from each other. However, at the end of the trial, rats in the normal control group recorded the highest percent weight gain, while those in the Vcr + Tau + Pca group had the lowest body weight gain, with their final body weight significantly ($P < 0.05$) lower than those of the other treated groups. The order of decreasing percent body weight gain was: N (31.04%) > Vcr + Tau (28.05%) > Vcr + Pau (19.18%) > Vcr (17.82%) > Vcr + Pca + Tau (15.11%). In comparison to control rats, the reduction in weight gain in other groups could be attributed to reduced feed consumption in these groups (data not shown).

The weights of the colon in the Vcr + Tau + Pca group was significantly lower than those of the other groups, probably reflecting the slower rate of body weight gain in this group (Table 1).

Effects of taurine and protocatechuic acid on Haematological indices in Vincristine-treated rats

The changes in haematological indices in the experimental rats are presented in Fig. 2. Results showed that administration of Vcr led to significant ($P < 0.05$) reduction in PCV (39.5%), RBC (41.1%), Hb (54.4%), WBC (67.6%) and platelet count (52.8%) when compared with the control. Similarly, the values of the haematological parameters in rats treated with Tau or Pca were also significantly ($P < 0.05$) lower than the control. However, Tau showed greater tendency for improvement of haematological parameters compared to Pca. In comparison with control, the percentage reductions in the values of the haematological parameters between Tau vs Pca were PCV (35.1% vs 44.0%); RBC (48.8% vs 59.1%); Hb (36.6% vs 46.3%); WBC (54.3% vs 68.3%); and Platelets (50.2% vs 56.2%). Interestingly, rats treated with the Vcr + Tau + Pca combination recorded the lowest values of haematological parameters among all the experimental groups. Compared to the control group, the Vcr + Tau + Pca group recorded drastic reductions in PCV (50.0%); RBC (52.5%); Hb (52.5%); WBC (79.5%) and Platelets (63.6%).

Effects of taurine and protocatechuic acid on bone marrow genotoxicity Vincristine-treated rats

The present study showed that treatment of rats with Vcr alone induced a highly significant ($P < 0.05$) increase in the frequency of micronucleated polychromatic erythrocytes (mnPCEs) compared with healthy control rats (Fig. 3). On the other hand, rats treated with Tau, Pca or their combination exhibited a highly significant ($P < 0.05$) reduction in the frequency of this chromosomal aberration.

Effects of taurine and protocatechuic acid on H_2O_2 generation and GSH concentration in liver, ileum and *colon* of Vincristine-treated rats

The effects of Tau and Pca administration on H_2O_2 and GSH concentrations in liver, ileum and colon are depicted in Fig. 4. Intraperitoneal administration of Vcr caused significant ($P < 0.05$) increase in H_2O_2 in the liver, ileum and colon, but significant ($P < 0.05$) reduction in GSH concentration in these tissues. However, when Tau and/or Pca were administered along with Vcr, there was significant ($P < 0.05$) reversal in the Vcr-induced changes i.e. reduction in H_2O_2 content in the liver, ileum and colon, while GSH level was significantly improved in the ileum and colon, especially with Tau administration.

Effects of taurine and protocatechuic acid on MDA and AOPP concentrations in liver, ileum and *colon* of Vincristine-treated rats

The levels of MDA and AOPP in the liver, ileum and colon are shown in Fig. 5. Compared to the control, rats treated with Vcr showed significant ($P < 0.05$) increase in MDA concentration in all the tissues examined. Levels of AOPP were also

Table 1 Effects of Taurine and Protocatechuic acid on body weights, liver and colon weights in vincristine-treated rats

	Control	*Vcr*	*Vcr + Tau*	*Vcr + Pca*	*Vcr + Tau + Pca*
Initial weight (g)	112.1 ± 14.93	112.8 ± 12.25	114.1 ± 9.71	116.3 ± 10.60	110.5 ± 17.995
Final weight (g)	146.9 ± 27.51	132.9 ± 18.27	146.1 ± 17.13	138.6 ± 19.98	127.2 ± 29.85[a]
Weight gain (%)	31.04	17.82	28.05	19.18	15.11
Liver weight (g)	6.28 ± 0.91	6.22 ± 0.83	6.87 ± 0.93	6.69 ± 0.92	5.35 ± 1.53
Colon weight (g)	1.09 ± 0.16	0.97 ± 0.15	0.94 ± 0.13	1.01 ± 0.25	0.70 ± 0.11[a,b,c,d]

%weight gain = {(final weight-initial weight)/initial weight} × 100; *Vcr* Vincristine, *Tau* Taurine, *Pca* Protocatechuic acid

[a]Significant ($P < 0.05$) as compared with control

[b]Significant ($P < 0.05$) as compared to Vcr group

[c]Significant ($P < 0.05$) as compared to Vcr + Tau group

[d]Significant ($P < 0.05$) as compared to Vcr + Pca group

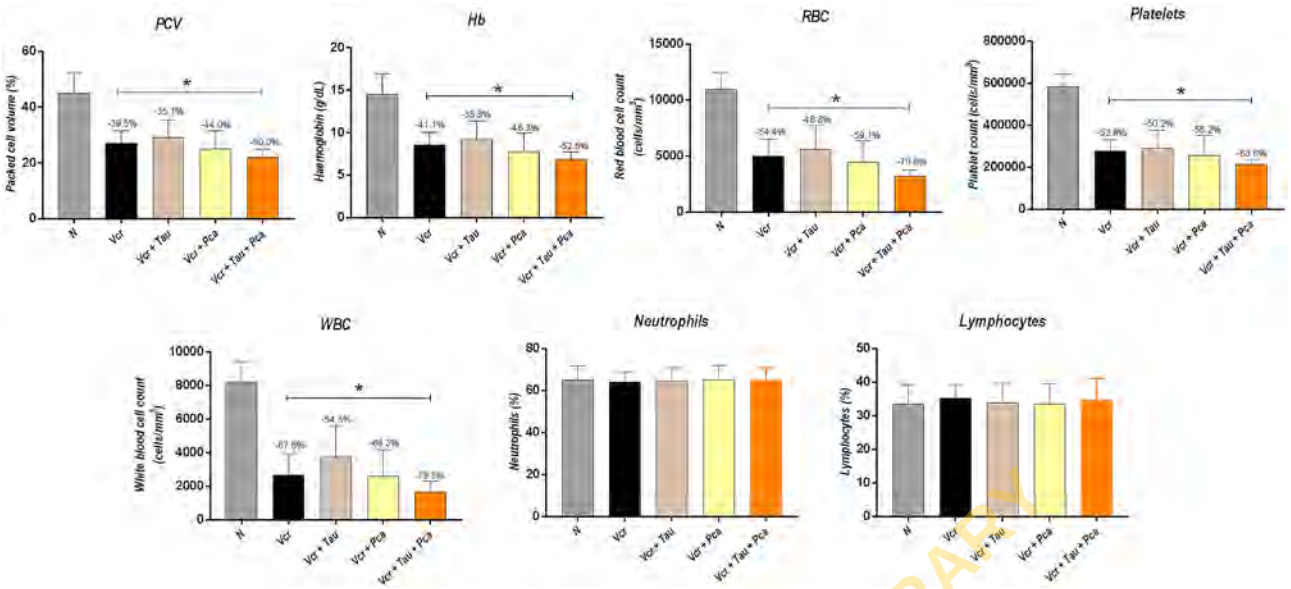

Fig. 2 Effects of taurine (Tau) and protocatechuic acid (Pca) on haematological parameters in Vincristine (Vcr)-treated rats (n = 10). Data are presented as mean ± standard deviation and analyzed using one-way ANOVA followed by Tukey's *post-hoc* analysis. * $P < 0.05$ as compared with control

significantly ($P < 0.05$) elevated in the ileum and colon of Vcr-treated rats, compared to the control. However, concurrent treatment of Vcr-treated rats with Tau, Pca or their combination produced significant ($P < 0.05$) reduction of MDA levels when compared to rats treated with Vcr alone. AOPP levels were only significantly ($P < 0.05$) ameliorated in the colon of rats treated with Tau or a combination of Tau and Pca, compared to Vcr-treated rats.

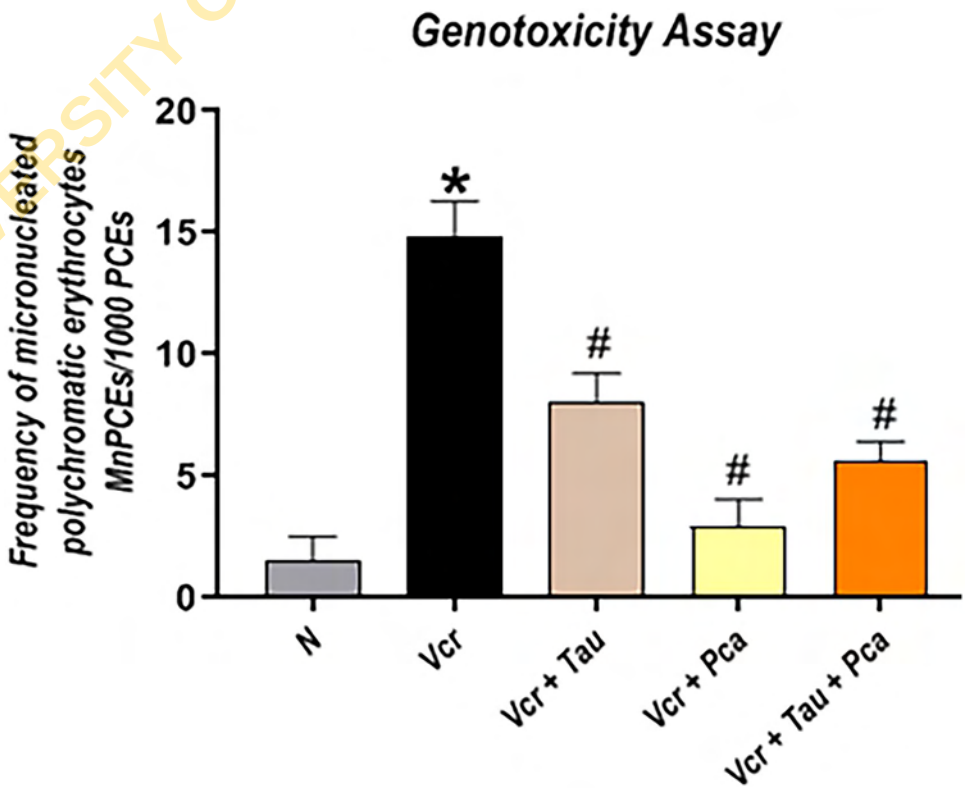

Fig. 3 Effects of taurine (Tau) and protocatechuic acid (Pca) on the proportion of micronucleated polychromatic erythrocytes in the bone marrow cells of Vincristine (Vcr)-treated rats (n = 10). Data are presented as mean ± standard deviation and analyzed using one-way ANOVA followed by Tukey's *post-hoc* analysis. * $P < 0.05$ as compared with control

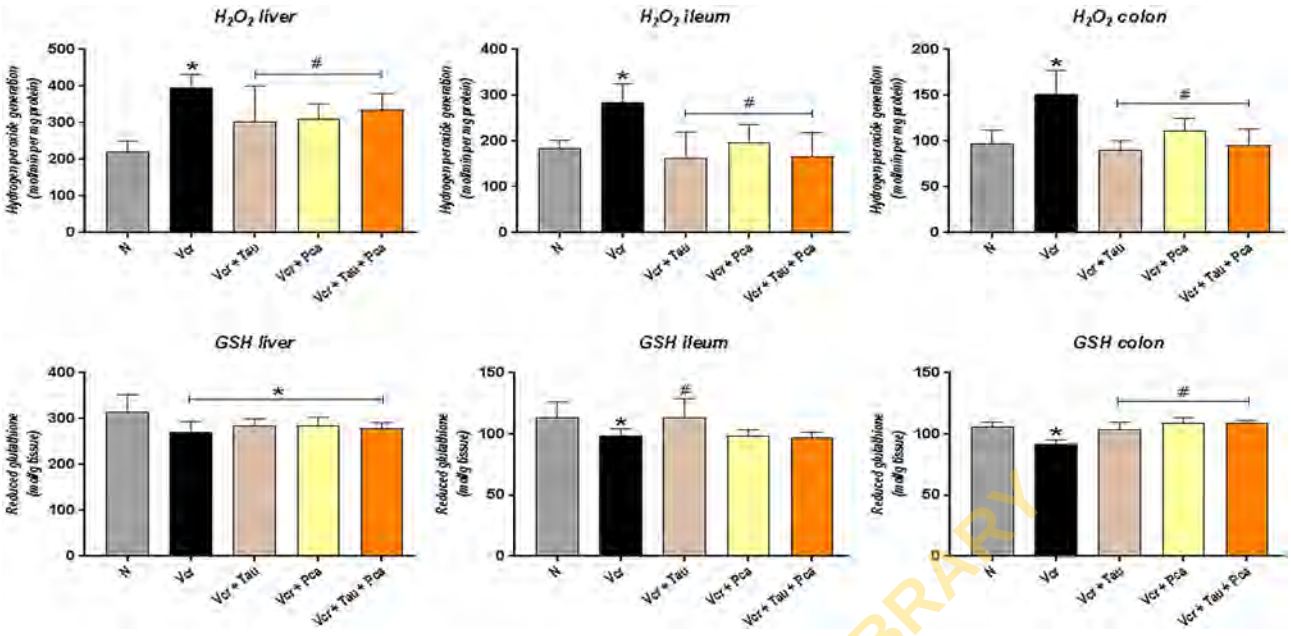

Fig. 4 Effects of taurine (Tau) and protocatechuic acid (Pca) on H_2O_2 and GSH concentrations in Vincristine (Vcr)-treated rats (n = 10). Data are presented as mean ± standard deviation and analyzed using one-way ANOVA followed by Tukey's *post-hoc* analysis. * $P < 0.05$ as compared with control; # $P < 0.05$ as compared with the Vcr group

Effects of taurine and protocatechuic acid on GPx and GST activities in liver, ileum and *colon* of Vincristine-treated rats

Vcr administration led to significant ($P < 0.05$) inhibition of GPx and GST activities in the liver, ileum and colon, compared to the control (Fig. 6). This result further reinforces the induction of oxidative stress and depletion of antioxidant capacity by Vcr in these tissues. Concurrent administration of Tau with Vcr led to the significant ($P < 0.05$) activation of GPx in all the tissues, as well as GST in the liver and colon, whereas, administration of Pca to Vcr-treated rats only produced significant

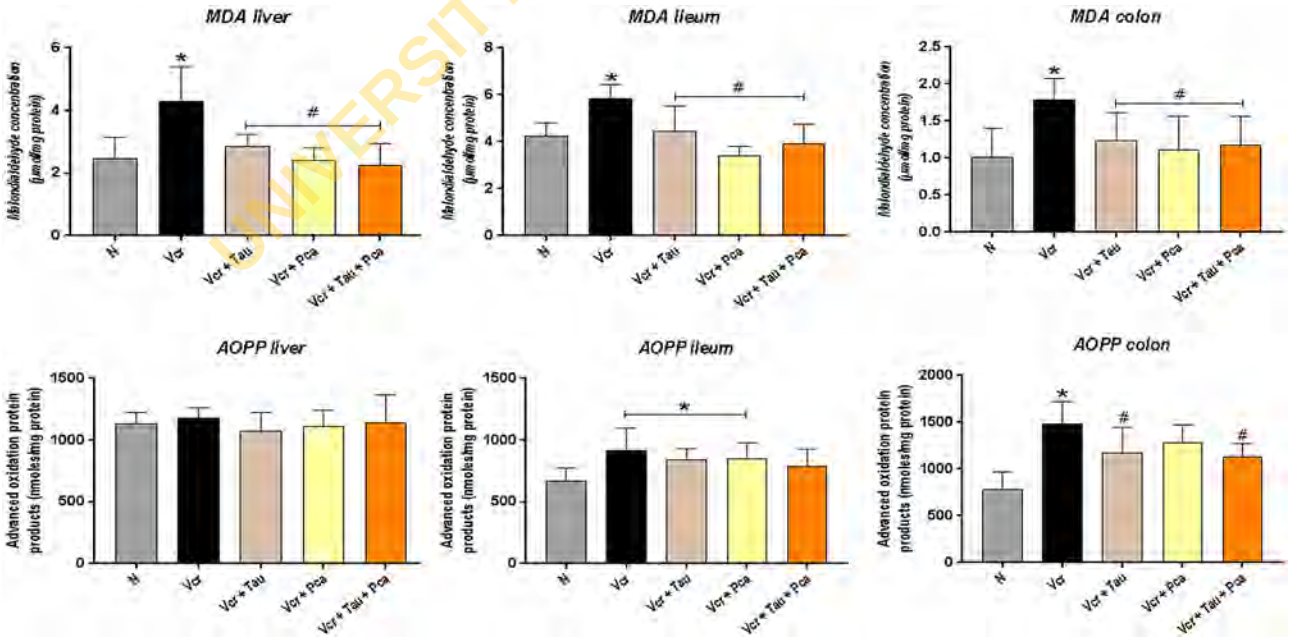

Fig. 5 Effects of taurine (Tau) and protocatechuic acid (Pca) on MDA and AOPP concentrations in Vincristine (Vcr)-treated rats (n = 10). Data are presented as mean ± standard deviation and analyzed using one-way ANOVA followed by Tukey's *post-hoc* analysis. * $P < 0.05$ as compared with control; # $P < 0.05$ as compared with the Vcr group

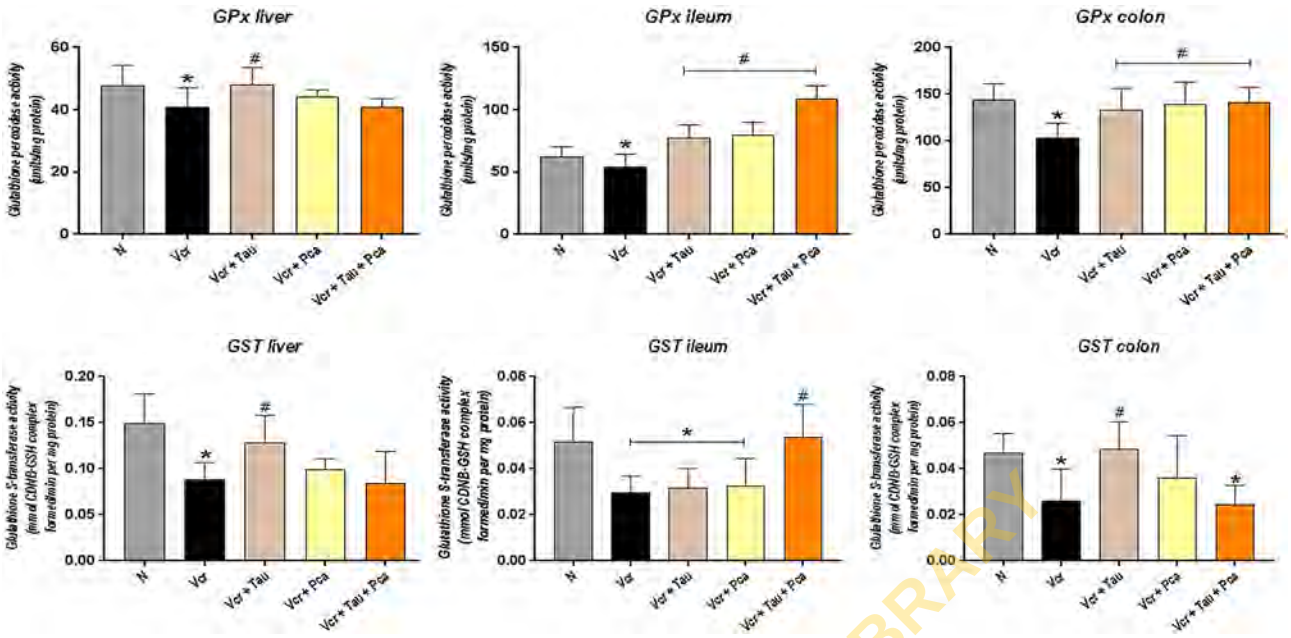

Fig. 6 Effects of taurine (Tau) and protocatechuic acid (Pca) on GPx and GST concentrations in Vincristine (Vcr)-treated rats (n = 10). Data are presented as mean ± standard deviation and analyzed using one-way ANOVA followed by Tukey's *post-hoc* analysis. * $P < 0.05$ as compared with control; # $P < 0.05$ as compared with the Vcr group

($P < 0.05$) improvement of GPx activity in the ileum and colon without any improvement in GST activity. In effect, it appeared that Tau administration was more effective than Pca as observed with GPx activity in the liver and GST activity in the liver and colon. Furthermore, treatment of Vcr-treated rats with a combination of Tau and Pca effectively improved GPx activity in the ileum and colon, as well as GST activity in the ileum.

Effects of taurine and protocatechuic acid on levels of TNF-α and IL-1β in serum of Vincristine-treated rats

The serum level of the pro-inflammatory cytokine TNFα was significantly ($P < 0.05$) elevated in the Vcr-treated rats, compared to the control (Fig. 7), while the level of IL-1β was not significantly affected. From the treatments administered, only the rats treated with Tau along with Vcr showed significant

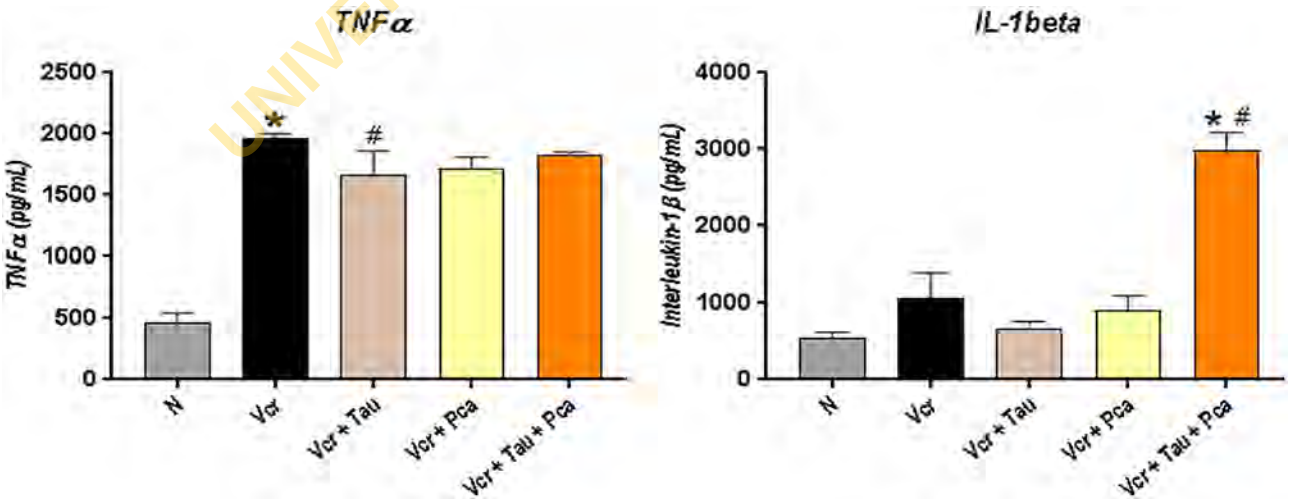

Fig. 7 Effect of taurine (Tau) and protocatechuic acid (Pca) on TNFα and IL-1β concentrations in serum of Vincristine (Vcr)-treated rats (n = 10). Data are presented as mean ± standard deviation and analyzed using one-way ANOVA followed by Tukey's *post-hoc* analysis. * $P < 0.05$ as compared with control; # $P < 0.05$ as compared with the Vcr group

Table 2 Effects of Taurine and Protocatechuic acid on serum enzymes in vincristine-treated rats

Parameter	Control	Vcr	Vcr + Tau	Vcr + Pca	Vcr + Tau + Pca
ALT (U/L)	23.63 ± 0.68	27.95 ± 1.64[a]	21.96 ± 0.76[b]	20.89 ± 5.41[b]	20.98 ± 4.67[b]
AST (U/L)	75.00 ± 7.07	104.73 ± 5.96[a]	81.58 ± 5.96[b]	77.63 ± 8.56[b]	86.05 ± 1.86[b]
ALP (U/L)	2.39 ± 0.50	5.52 ± 0.04[a]	2.45 ± 0.53[b]	4.14 ± 0.53[b]	2.76 ± 0.07[b]

Data are presented as mean ± standard deviation and analyzed using one-way ANOVA followed by Tukey's post-hoc analysis

[a]Significant when compared with control

[b]Significant when compared to Vcr group

($P < 0.05$) lowering of TNFα, while co-administration of Vcr with Pca or Tau + Pca failed to produce significant reversal of Vcr-induced TNFα increase. Although treatments with either Tau or Pca did not alter serum IL-1β levels as compared to control values, rats in the Vcr + Tau + Pca group presented a dramatic rise in the serum levels of this cytokine to levels significantly ($P < 0.05$) higher than those of all the other groups.

Serum activities of Liver enzymes in rats treated with Vincristine, Taurine and Protocatechuic acid

As shown in Table 2, Vcr administration led to significant ($P < 0.05$) increase in the serum activities of ALT, AST and ALP as compared to the control. In contrast, the serum activities of ALT, AST and ALP were significantly ($P < 0.05$) lowered in the Vcr + Tau, Vcr + Pca and Vcr + Tau + Pca groups.

Effects of taurine and protocatechuic acid on histological alterations in liver, ileum and *colon* of Vincristine-treated rats

Liver histology

The morphology of the liver parenchyma was investigated by staining with haematoxylin and eosin and the representative photomicrographs are presented in Fig. 8. The liver from untreated control rats showed normal central venules, hepatocytes and sinusoids without inflammatory cell infiltration. In Vcr-treated rats, some areas of necrotic parenchyma were found surrounding the central venules as well as mild inflammatory cell infiltration. Rats treated with Tau, Pca or Tau + Pca combination, however, showed generally well-preserved liver morphology with normal central venules, hepatocytes and sinusoids.

Ileum histology

The representative micrographs of the ileal mucosa after the different treatments are depicted in Fig. 9. The control rats presented well preserved mucosal epithelium with normal villi. The lamina propria and the glands showed no infiltration of inflammatory cells. The sub mucosal and muscularis layers also appeared normal. Following Vcr administration, however, rats showed poorly preserved mucosal epithelium with some sloughed villi, while the lamina propria and the glands show severe infiltration of inflammatory cells. The sub mucosal layer also appeared mildy infiltrated. Rats in the Vcr + Tau and Vcr + Pca groups showed well preserved ileal mucosal epithelium with normal villi, although the lamina propria and the glands showed moderate infiltration of inflammatory cells. The sub mucosal and muscularis layer, however had normal morphology. The ileum in the Vcr + Tau + Pca groups showed well preserved mucosal

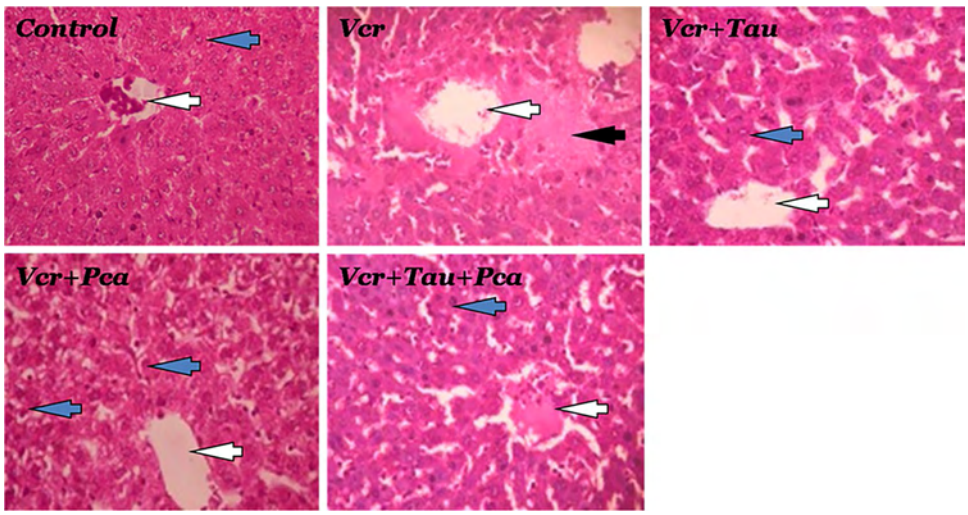

Fig. 8 Photomicrographs showing general morphology of liver sections stained with H&E; ×400. Control rats showed normal central venules (white arrow), hepatocytes (blue arrow) and sinusoids; Vcr-treated rats showed areas of necrosis around the central venules (black arrow) with a mild degree of inflammatory cell infiltration; Rats from the Vcr + Tau, Vcr + Pca and Vcr + Tau + Pau groups had normal hepatic morphology including normal central venules, hepatocytes and sinusoids

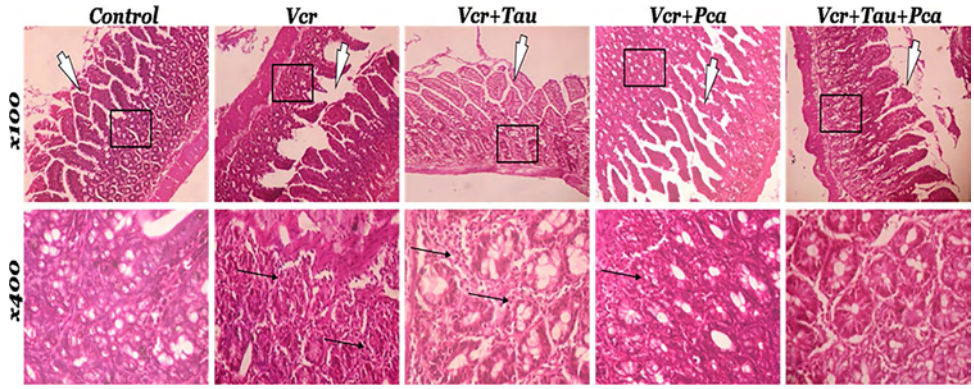

Fig. 9 Photomicrographs showing general morphology of sections of the ileum stained with H&E. Upper plates (Mag. × 100), lower plates (Mag. × 400). Control rats showed well preserved mucosal epithelium (white arrow), submucosa and muscular layer, normal villi and glands with no inflammatory cell infiltration; The Vcr-treated rats exhibited poorly preserved mucosa with evidence of sloughed villi (white arrow) and severe inflammatory cell infiltration (black slender arrow); In the Vcr + Tau, Vcr + Pca and Vcr + Tau + Pca groups, rats showed moderately well preserved mucosal epithelium (white arrows), normal villi and mild to moderate inflammatory cell infiltration (black slender arrows). The muscular layer, however, appears normal

epithelium with normal villi, absence of inflammatory cell infiltration in the lamina propria, glands and sub mucosa layer while the muscularis layer also appeared normal.

Colon histology

As shown in Fig. 10, the colonic sections from control rats showed well-preserved mucosal and sub-mucosal layers as well as absence of inflammatory cell infiltration. However, in the colon of rats treated with Vcr, there was evidence of moderate mucosal ulceration with moderate to severe infiltration of inflammatory cells into the mucosa and submucosa. However, in rats treated with Tau, and/or Pca, the rats' colon showed well-preserved mucosal epithelium and only mild degrees of inflammatory cell infiltration in the submucosa.

Immunohistochemical analysis of protein expression levels of Cytochrome c, Bax and Bcl-2 in the ileum and liver

The protein expression levels of Cytochrome c, Bax and Bcl-2 in the ileum and liver were analyzed by immunohistochemistry (Figs. 11 – 13). As shown in Fig. 11A and B, the protein expression level of Cyt c in rats administered Vcr was up-regulated by 43.55% and 17.47% in the ileum ($P < 0.05$) and liver, respectively, relative to control rats. However, in comparison with the Vcr group, treatment with Tau, Pca or Tau/Pca combination caused down-regulation of Cyt c by 13.89%, 22.58% and 29.99%, respectively in the ileum and by 23.54%, 10.61% and 34.01% in the liver.

The protein expression level of Bax in the ileum and liver is shown in Fig. 12A and B. Following

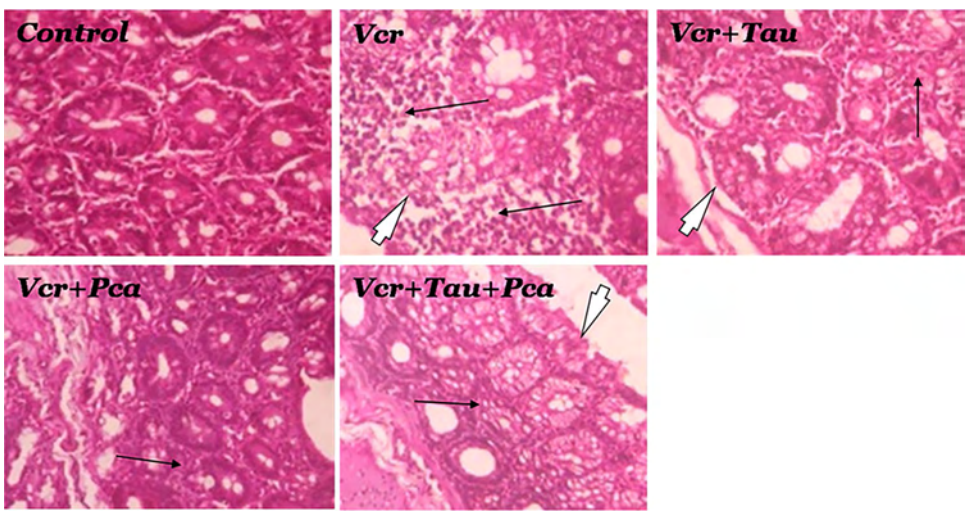

Fig. 10 Photomicrographs showing general morphology of sections of the ileum stained with H&E; × 400. Control rats had well preserved mucosa and submucosa layers with no evidence of inflammatory cell infiltration; Vcr-treated rats showed moderate degrees of mucosal ulceration (white arrow) with severe inflammatory cell infiltration (black slender arrows); the mucosal epithelium was well preserved in the groups treated with Tau and/or Pca, albeit with mild sub-mucosal inflammatory cell infiltration (black arrows)

administration of Vcr, the protein expression level of Bax was up-regulated by 33.92% and 52.77% in the ileum and liver ($P < 0.05$), respectively, in comparison with the control. Compared with the rats treated with Vcr, however, administration of Tau, Pca or Pca/Tau produced down-regulation of Bax in the ileum by 35.26%, 36.07 and 20.85%, respectively, while liver Bax protein expression level decreased by 27.22%, 29.40% and 38.16% following administration of Tau, Pca and Tau/Pca, respectively.

Immuno-histochemical expression of Bcl-2 in the ileum and liver is depicted in Fig. 13A and B, respectively. When compared with healthy controls, Vcr administration produced a down-regulation of Bcl-2 expression by 31.59% and 14.26% in the ileum and liver, respectively. This effect was reversed in the ileum of treated rats where the rats recorded an up-regulation in Bcl-2 expression up to 53.57%, 46.19% and 52.625 in Tau, Pca and Tau + Pca-treated rats, respectively, when compared with Vcr-treated rats. Similar up-regulation of Bcl-2 expression in the liver of these rats in relation to Vcr-treated rats

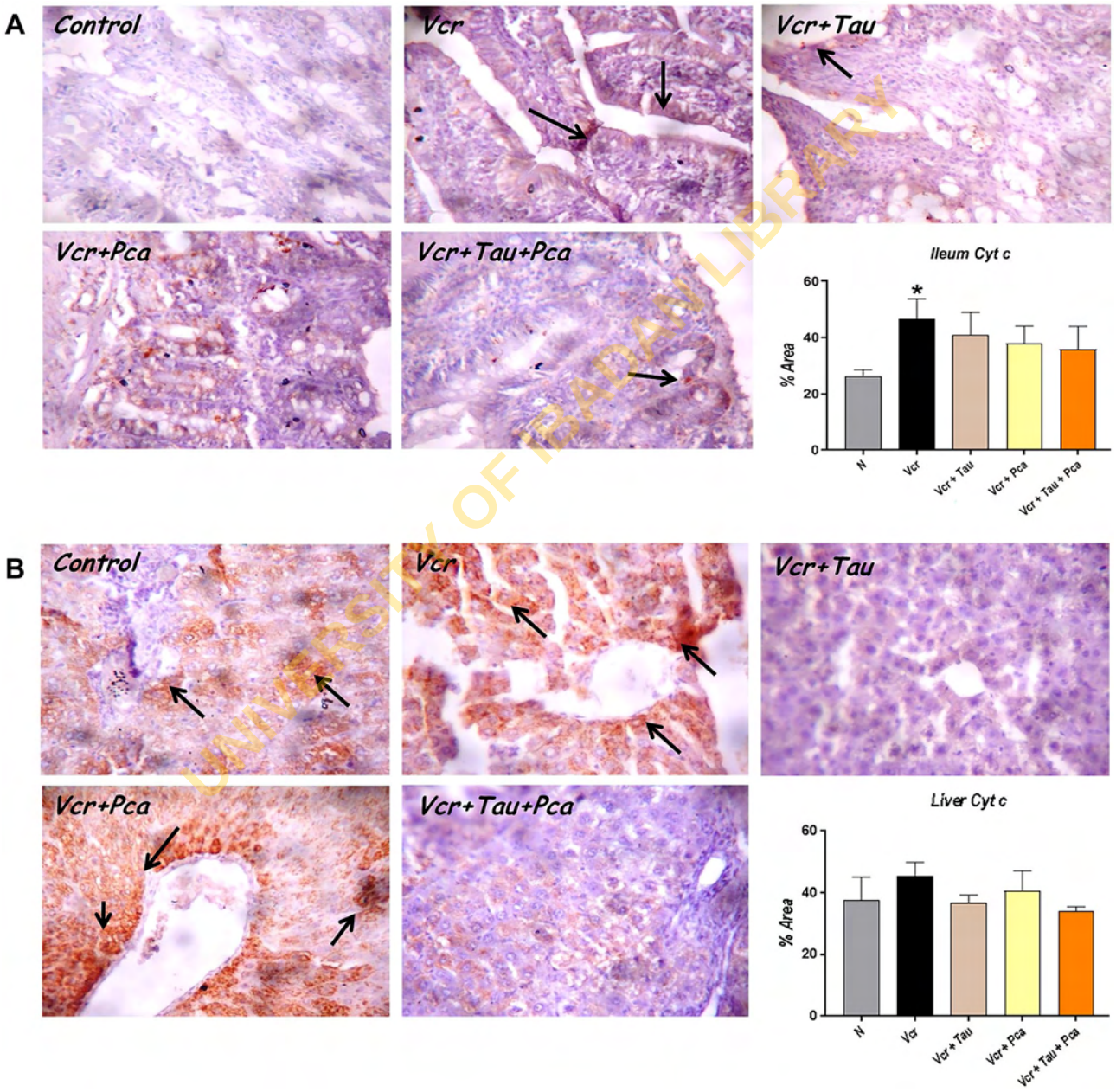

Fig. 11 Immunohistochemical analysis of the expression of Cytochrome c in the (**A**) ileum and (**B**) liver of rats treated with Vincristine (Vcr), Taurine (Tau) and/or Protocatechuic acid (Pca). Bars represent the semi-quantitative values (mean ± SD; n = 6) of % area of cytochrome c (Cyt c) expression. * indicates statistically significant difference ($P < 0.05$) between the control group and the Vcr group

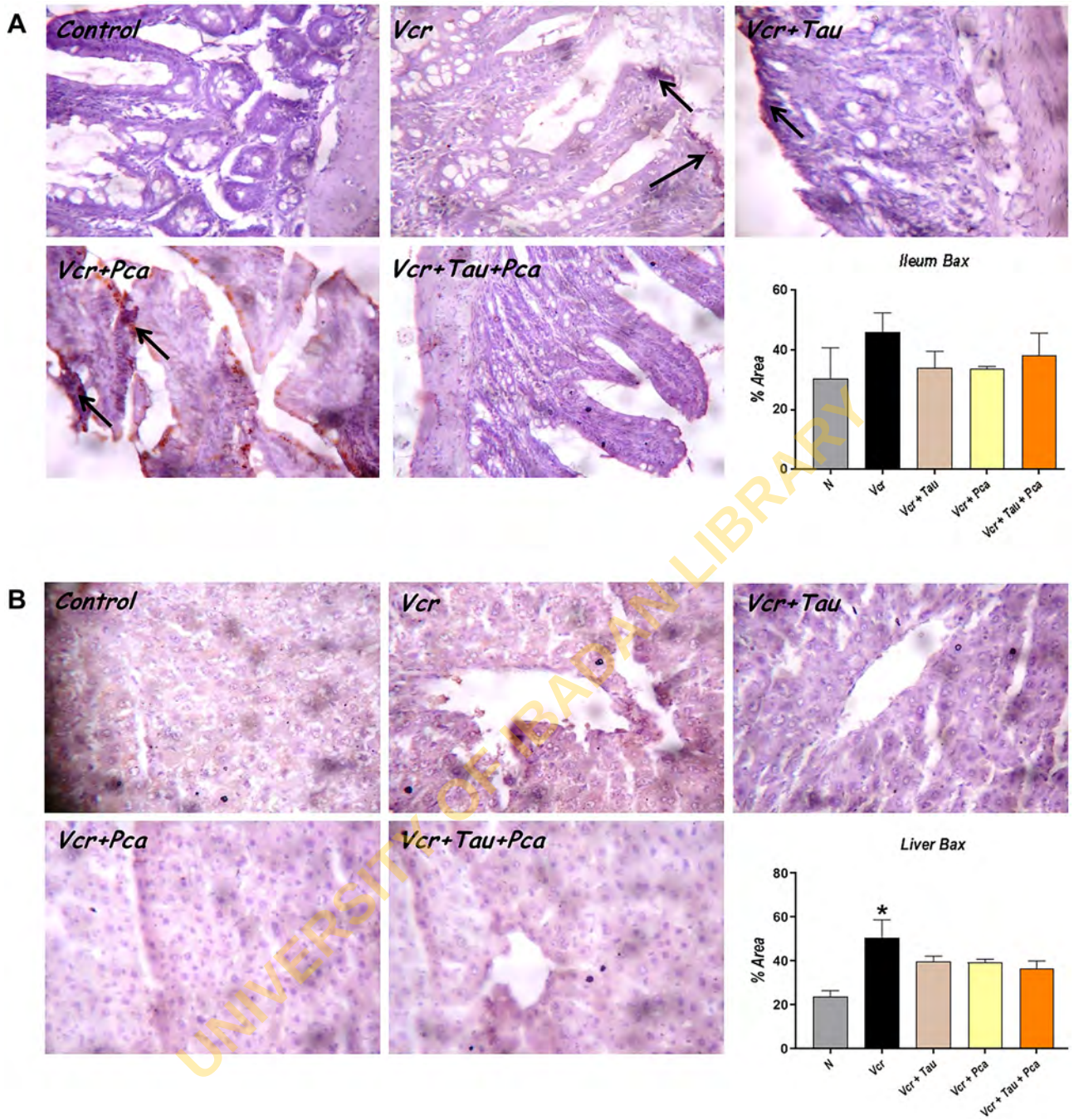

Fig. 12 Immunohistochemical analysis of the expression of Bax in the (**A**) ileum and (**B**) liver of rats treated with Vincristine (Vcr), Taurine (Tau) and/or Protocatechuic acid (Pca). Bars represent the semi-quantitative values (mean ± SD; n = 6) of % area of Bax expression * indicates statistically significant difference ($P < 0.05$) between the control group and the Vcr group

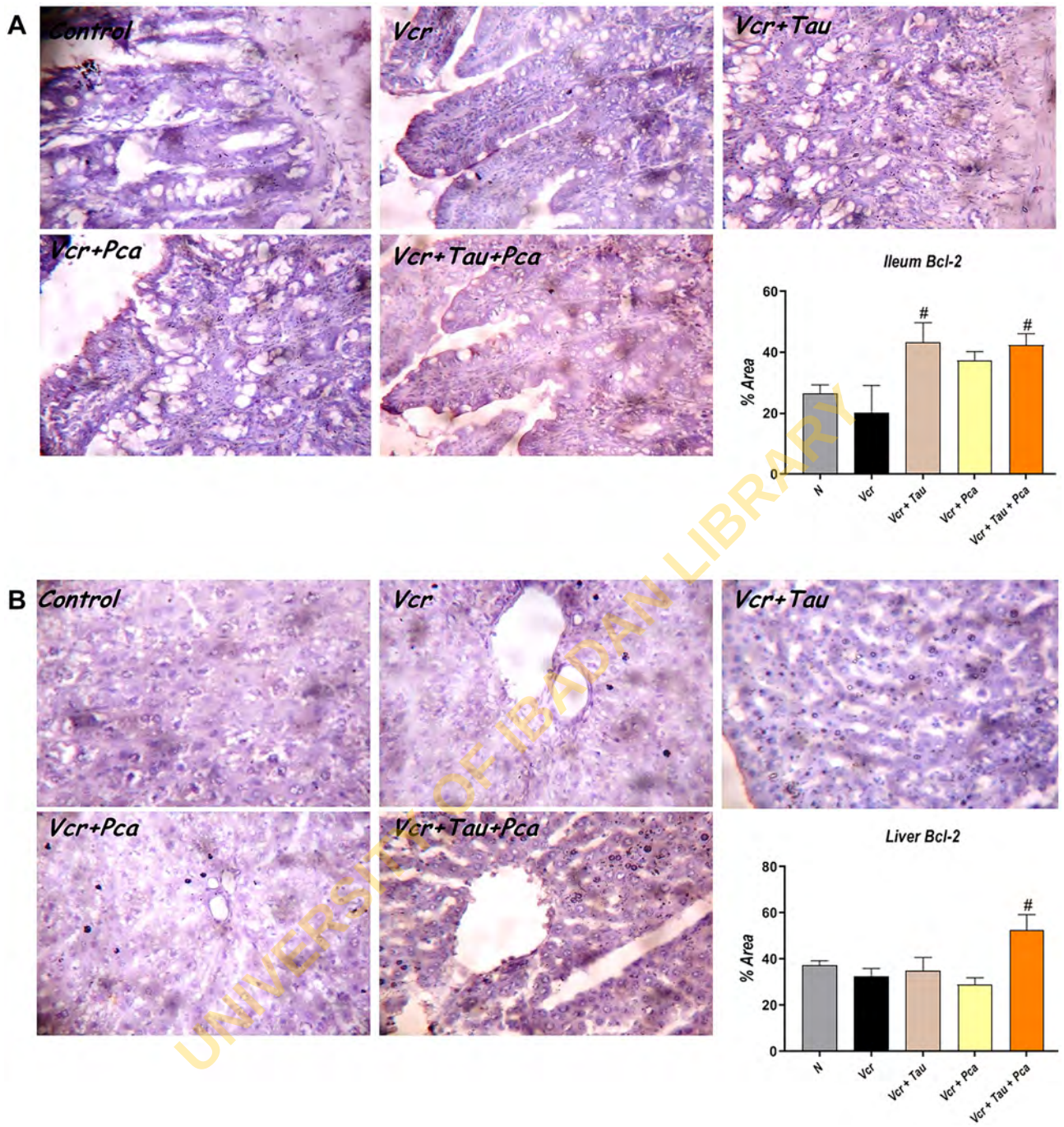

Fig. 13 Immunohistochemical analysis of the expression of Bcl-2 in the (**A**) ileum and (**B**) liver of rats treated with Vincristine (Vcr), Taurine (Tau) and/or Protocatechuic acid (Pca). Bars represent the semi-quantitative values (mean ± SD; n = 6) of % area of Bcl-2 expression # indicates statistically significant difference ($P < 0.05$) between the control group and the Vcr group

revealed an increase of 6.09% and 37.87% in the Vcr + Tau and Vcr + Tau + Pca groups, respectively.

Discussion

Chemotherapy with Vcr sulphate is associated with several side effects including gastrointestinal complications such as constipation, diarrhea, abdominal cramps, as well as bone marrow suppression, hepatotoxicity and neurotoxicity (Herradón et al. 2021). In the present study, we hypothesized that treatment with Tau, Pca or their combination could produce alleviation of toxic effects in the liver, intestines and bone marrow of Vcr-exposed rats. The findings indicated that treatment of Vcr-exposed rats with either Tau or Pca alone resulted in significant amelioration of tissue damage via inhibition of oxidative and inflammatory processes in the selected tissues. In addition, an inhibition of apoptosis also appeared to play a significant role in the protection exerted by these compounds in the liver and ileum. Despite profound evidence of local tissue protection when the animals were treated with Tau or Pca, our results indicated that combining these compounds for Vcr treatment may require caution as data on some systemic indices point to the induction of stress mechanisms in the group of rats given the Vcr + Tau + Pca combination, compared to when these compounds were administered separately.

Body and organ weights are important endpoints in the assessment of the toxicity of chemicals and other potentially harmful substances (Bailey et al. 2004). In this study, Vcr administration with or without Tau and/or Pca treatment caused slowing of body weight gain, as indicated by reduced body weight gain in these groups of rats when compared with control. Our findings agree with previous studies by (Li et al. 2021) which reported that Vcr treatment significantly slowed body weight gain in both obese and lean rats. Although, the average weights of the animals in all the groups increased after the experimental period, the percentage weight gain was significantly lower in rats from the Vcr + Tau + Pca group, when compared with all other groups. In addition, the average colon weight in this group was also significantly lesser than those of other groups.

The inability of rats in the Vcr + Tau + Pca group to gain weight as much as rats in the other groups gave a first indication of possible detrimental effect of using these drugs simultaneously. Since the initial weights of animals in the various groups were not statistically different, it was more likely that an obvious reduction in food and water intake by the *ad-libitum* fed animals (data not shown), probably due to an induced lack of appetite, was a major contributor to the decline in body weight of the rats witnessed in this group. Reduction in food intake and consequent weight loss has been attributed to the production of the hormone leptin which is mainly produced from the adipose tissue, stomach and brain and serves to provide feedback to hypothalamic centers that regulate hunger and satiety (Farooqi et al. 2001; Gemmill et al. 2003).

The general reduction in haematological parameters (PCV, Hb, RBC, WBC and platelet count) obtained in this study is an indication of myelosuppression caused by Vcr administration. Myelosuppression is often a common side effect of majority of anticancer drugs (Wang et al. 2006). The present result agrees with (Rocha et al. 2019) who also reported significantly lower values of RBC and WBC in Vcr-treated rats. In the present study, only Tau administration produced marginal reversal of the haematological alterations, in comparison to Vcr-treated rats, while Pca treatment was ineffective in this regard. This result suggests that the two compounds were unable to significantly improve the mitotic index of the bone marrow cells in order to effectively counteract the myelosuppression induced by Vcr. Contrary to expectations, however, treatment of Vcr-exposed rats with a combination of Tau at 50 mg/kg and Pca at 50 mg/kg resulted in the worsening of haematological alterations induced by Vcr. Again, the depression of haematology variables in this group appear to corroborate the data on the body weights of the rats, suggesting that drug combinations involving simultaneous administration of Tau, Pca and Vcr may have detrimental effects on general physiological indices and should, therefore, be considered with caution. As better results were obtained with separate Tau or Pca administration with Vcr, the possibility of negative drug interactions between Tau and Pca needs to be investigated in future studies.

In order to further understand the potential protective roles of Tau and Pca on blood-forming tissues, we investigated their effects on bone marrow genotoxicity induced by Vcr. While Vcr exposure led to a highly significant increase in the appearance of micronucleated erythrocytes, treatment with Tau, Pca or their combination all produced significant inhibition of chromosomal changes that result in micronuclei formation. This anti-clastogenic activity of Tau and Pca may help to reduce the incidence of secondary tumors and infections in cancer patients undergoing chemotherapy (Ramadan et al. 2012). It is noteworthy that only few in vivo studies have assessed the bone marrow anti-clastogenic effects of Tau as a single compound, with a report claiming its anti-genotoxic effect as component of a plant juice containing several other compounds (Madrigal-Santillán et al. 2013). Another study revealed the reduction of micronuclei by Tau in Aluminum Sulphate-Induced DNA Damage in Human Peripheral Lymphocytes (Türkez and Geyikoğlu 2010). Studies have also shown that Pca, an anthocyanin-derived phenolic compound, possesses anti-genotoxic and

chemo-preventive properties probably exerted via its antioxidant, anti-inflammatory, anti-apoptotic activities (Kakkar and Bais 2014; Habib et al. 2021).

The contributory role of ROS to Vcr-induced tissue damage is being increasingly investigated (Alenezi 2023). The intestines are particularly prone to attack from xenobiotic-induced ROS arising from the hypoxanthine/xanthine oxidase and NADPH oxidase systems which produce large amounts of superoxide radicals (Martin et al. 2004). Excessive ROS generation stimulates damage to major tissue macromolecules and potentially induces inflammation via activation of polymorphonuclear leucocytes with further increase in tissue damage (Bhattacharyya et al. 2014). In this study, our data revealed that Vcr administration led to accumulation of H_2O_2 in the liver, ileum and colon, along with increased MDA levels in the same tissues and increased AOPP levels in the intestines. However, GSH level was down-regulated, while GPx and GST activities were inhibited by Vcr. Treatment with Tau or Pca as well as their combination caused alleviation of oxidative stress as the levels of H_2O_2 and MDA were reduced in all the tissues and AOPP level was reduced in the colon. The results showed that Tau demonstrated greater antioxidant activity compared to Pca as Tau administration effectively stimulated GSH, GPx and GST in virtually all the tissues, whereas Pca was mostly effective in the colon. When administered together, both compounds exerted synergistic antioxidant actions in all the tissues. The results are in line with previous studies including excellent reviews on the antioxidant actions of Tau and Pca (Surai et al. 2021; Mert et al. 2022; Widy-Tyszkiewicz 2022).

Increased pro-inflammatory cytokine (TNFα) was detected in the serum of animals given Vcr alone, while Tau administration, among other treatments, caused significant amelioration of the systemic elevation of TNFα. We observed significantly elevated serum levels of IL-1β in rats treated with the Vcr + Tau + Pca combination, which otherwise remained unaltered in other groups compared to control rats. We attribute the observed increase in the interleukin to the possibility of profound hypoglycaemic stress due to a noticeable decline in feed intake by rats from this group. Although the measurement of blood glucose levels was not undertaken in the present study, previous studies have shown that hypoglycaemia, such as that induced by insulin in non-diabetic subjects, is associated with increase in pro-inflammatory cytokines including TNFα, IL-1β, IL-6 and IL-8 (Razavi et al. 2009). This effect may also be related to the observed decline haematological indices and body weight earlier noted in animals from this group, and reinforces the suggestion that the compounds Tau and Pca are better suited to individual usage rather than being combined. Although little is known about the toxicity of Tau/Pca combinations, previous studies indicated that Tau co-administration with ethanol was toxic, and in some cases, lethal to mice, while a marked fall in blood glucose was also thought to be the main reason for lethality (Taranukhin et al. 2013). Notably, the authors reported that all mice treated separately with either Tau or ethanol survived and had normal blood glucose levels.

Despite some evidence of systemic toxicity in the Vcr + Tau + Pca group, the rats showed reduced activities of serum enzymes, ALT, AST and ALP indicating significant local protection liver tissues. The protection was also corroborated by a preservation of the liver parenchyma and hepatocytes from drug-induced injury. Similar protection was also obtained in rats treated separately with either Tau or Pca, unlike hepatocytes from the Vcr group which showed areas of necrosis especially around central venules. Upon histological examination of the intestines, the predominant lesions observed include infiltration of inflammatory cells into the intestinal mucosa as well as erosions and ulcerations of the ileal and colonic epithelium. To a large extent, these lesions were alleviated in rats administered with Tau and/or Pca. The tissue protection is attributable to the already described antioxidant and anti-inflammatory activities of these two compounds.

Drug-induced cell death is a major feature of treatment with most chemotherapeutic drugs and this may involve intrinsic (mitochondrial-regulated) or extrinsic (death receptor-mediated) pathways of apoptosis. In the present study, emphasis was placed on investigation of the mitochondrial pathway with immunohistochemical staining showing increased expressions of cytochrome c and pro-apoptotic Bax in the ileum and liver, while the expression of anti-apoptotic Bcl-2 was down-regulated. Apoptosis is initiated by the release of cytochrome c from mitochondrial membranes which is normally triggered by the interaction of pro-apoptotic members of the Bcl-2 family, in particular Bax (Milner et al. 2002). It appears from the present findings that the up-regulation of Bcl-2 and a corresponding down-regulation of cytochrome c and Bax may be partially responsible for the protection of liver and intestinal cells. This anti-apoptotic effect could also have played a role in the protection of bone marrow cells from genotoxicity induced by Vcr. Since cancer therapy is usually limited by toxicity to normal cells, selective killing of cancer cells is being explored by using antagonistic drug combinations, which include cytotoxic and protective drugs, as is being investigated in the present study. The challenge, though, is ensuring that the protection of normal cells does not also lead to anti-cancer drug resistance in cancer cells (Blagosklonny 2023). Striking the right balance is surely the new goal of effective cancer management involving chemotherapeutic drugs.

We identified a few limitations in the present study which should be improved upon in future studies. First, our study did not include measurement of blood glucose levels which

could have guided the interpretation of our data considering the aggravation of some systemic alterations, including blood and immunological indices when rats were treated with all three compounds i.e. Vcr, Tau and Pca, simultaneously. This measurement should be considered necessary for future studies because of the propensity for induction of anorexia and possible hypoglycaemic stress by the three drugs used in this study (Bae et al. 2022; D'Archivio et al. 2018). Second, this study focused on doses of Tau and Pca that have been considered therapeutic in previous studies which did not allow the assessment of probable dose-associated differences in the efficacies of each drug against Vcr toxicities. Therefore, future studies should consider the testing of lower or higher doses.

Comparatively, our data showed a relatively greater efficacy of Tau over Pca in alleviating Vcr-induced toxicity. The greater efficacy of Tau against Vcr toxicity, as compared to Pca, could be explained in the light of a possible increase in its blood levels arising from the activation of its de novo synthesis from L-cysteine via the taurine biosynthetic pathway in the liver which involves the enzyme cysteine dioxygenase (CDO) (Hirschberger et al. 2001). The combined treatment of rats with Tau and Pau produced greater degree of local protection of tissues probably as a result of synergistic antioxidant effects of the two compounds. However, our data showed that the strategy may be limited considering the likelihood of aggravating Vcr-induced alterations in some physiological parameters including blood and immunological indices.

In conclusion, treatment of rats with taurine and/or protocatechuic acid significantly alleviated Vincristine-induced toxicity to the liver, bone marrow and intestines through mechanisms that involved antioxidant, anti-inflammatory and anti-apoptotic activities. However, when used separately, rather than combined, each drug may be useful as adjuvant therapy for reducing genotoxicity, hepatotoxicity and intestinal toxicity in patients undergoing chemotherapeutic interventions.

Declarations

Funding This study was not supported by any funding.

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Not applicable

Consent for publication Not applicable

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