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Immunomodulatory Activity of Propyl Gallate Against Cyclophosphamide Induced Immunosuppression in Rats

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ABSTRACT

Background: Cyclophosphamide (CP) is a cytotoxic drug with immunosuppressive properties that is used extensively; its clinical value is limited by bone-marrow suppression and damage to lymphoid organs. Propyl gallate (PG), the n-propyl ester of gallic acid, is a phenolic antioxidant reported to have anti-inflammatory and cytoprotective properties, although no report has addressed its influence on CP-induced immune injury. Methods: Wistar rats were divided into five groups: normal control, CP control, levamisole (2.5 mg/kg) + CP, PG 30 mg/kg + CP and PG 60 mg/kg + CP. Test agents were administered for 14 days, with CP (80 mg/kg, i.p.) injected over the last 7 days. End-points comprised blood counts, spleen and thymus indices, carbon-clearance phagocytic index, neutrophil adhesion, splenic natural killer (NK) cell cytotoxicity, serum IgG and IgM (ELISA), and haematoxylin–eosin histology of the spleen and thymus. Results: CP reduced WBC, RBC and platelet counts by 50.5%, 37.0% and 27.0% respectively, approximately halved the spleen and thymus indices, and impaired phagocytosis, NK-cell cytotoxicity and immunoglobulin levels. Compared with CP alone, PG at 60 mg/kg raised WBC (+63%), RBC (+23%) and platelets (+20%), improved the spleen and thymus indices, brought phagocytic capacity back to normal, recovered NK-cell cytotoxicity (83.7% versus 63.4%) and elevated IgG and IgM by 55% and 67%. The 30 mg/kg dose gave smaller and mostly partial responses, with evident improvement in leukocyte counts, immune organ indices, phagocytosis, NK activity and immunoglobulins. Histological examination indicated that PG maintained lymphoid cellularity in the spleen and thymus. Conclusion: In a dose-related manner, PG shielded the haematopoietic, lymphoid and effector-cell compartments from CP, with an effect size close to that of levamisole. These results mark PG as a candidate immunorestorative adjunct worthy of mechanistic, safety and tumour-model investigation..

Introduction

The liver occupies a central position in xenobiotic metabolism, and this metabolic burden renders it particularly susceptible to injury from a wide range of therapeutic agents. Drug-induced liver injury remains one of the most frequent causes of hepatic dysfunction encountered in clinical practice and is a recurring concern during the development and long-term use of several classes of drugs, including antineoplastic and immunomodulatory agents [1–3].

Host defence rests on the joint action of two arms. The innate arm responds within hours through epithelial barriers, phagocytes, dendritic cells, NK cells and soluble mediators; the adaptive arm, driven by T and B lymphocytes, is antigen-specific and retains a memory of earlier exposures [1–3]. IgM and IgG antibodies connect the two and take part in neutralisation, opsonisation and complement activation [4]. As the thymus and spleen produce, educate and harbour lymphocytes, their relative weight and cellularity are widely used as crude measures of immune competence.

Controlled suppression of immunity is essential for transplantation and for the management of autoimmune disease. The agents employed, among them calcineurin inhibitors, antimetabolites, corticosteroids, antibody preparations and cytotoxic drugs, compromise host defence, raising the risk of infection and cancer, and some also cause organ-specific toxicity [5,6]. The arrival of cyclosporin A in the 1970s exemplifies both the advantages and the cost of this strategy [6,7].

Cyclophosphamide (CP) is an alkylating drug prescribed for numerous cancers and, at reduced doses, for autoimmune disorders and for conditioning before marrow transplantation [8,9]. As a prodrug it is activated by hepatic cytochrome P450 enzymes; the phosphoramide mustard formed cross-links the DNA of dividing cells, and acrolein contributes further oxidative tissue damage [10,11]. Because marrow progenitors and lymphocytes divide rapidly, they are especially vulnerable, and CP

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therefore causes pancytopenia, thymic and splenic atrophy, and suppression of both cellular and humoral immunity [12–14]. The reproducibility of these effects has made CP-treated rodents a standard system for evaluating immunorestorative agents, and extracts, polysaccharides, peptides and purified phytochemicals have all been tested in it [15–26].

Propyl gallate (PG; propyl 3,4,5-trihydroxybenzoate), the n-propyl ester of gallic acid, is a phenolic antioxidant added to fats, oils and cosmetics to prevent oxidative deterioration [27,28]. Its phenolic hydroxyl groups are able to donate hydrogen atoms to lipid peroxyl and other radicals, and calculations predict effective radical scavenging in aqueous as well as lipid environments [29]. Experimental reports credit PG with limiting myocardial oxidative injury [30], suppressing NF-κB-dependent inflammatory signalling [27,31], attenuating neuroinflammation [32], restraining adipogenesis [33] and protecting hepatocytes via effects on ferroptosis [34]. It also modulates ROS-dependent apoptosis and, in certain tumour cells, behaves as a pro-oxidant [35,36].hydroxyl groups can donate hydrogen atoms to lipid peroxyl and other radicals, and computational work predicts efficient radical scavenging in both aqueous and lipid media [29]. In experimental systems PG has been reported to limit myocardial oxidative injury [30], suppress NF-κB-dependent inflammatory signalling [27,31], reduce neuroinflammation [32], restrain adipogenesis [33] and protect hepatocytes through effects on ferroptosis [34]. PG also alters ROS-dependent apoptosis, and in some tumour cells it acts as a pro-oxidant [35,36].

Since oxidative stress and inflammatory signalling contribute to CP toxicity, PG is a plausible protective candidate, but its effect on CP-induced immunosuppression has not been studied. We accordingly compared two oral doses of PG against the reference immunostimulant levamisole in CP-treated Wistar rats, using haematological, organ-weight, innate-effector, humoral and histological end-points.

Materials and methods

Animals and Ethical Approval

Adult albino Wistar rats (3 months old, 180–250 g) of both sexes were sourced from the institutional animal facility. Housing was in nylon-micro cages at 25 ± 2 °C with a 12 h light/dark cycle, standard laboratory diet and water ad libitum. Males and females were housed separately, and all animals underwent quarantine and 15 days of acclimatisation before the experiment began. The Institutional Animal Ethics Committee approved the study protocol (IAEC/R.MUKESHKANNA/TNMGRU/M.PHARM/261624500506). [16 animals are used.]

Test Agents and Doses

CP was dissolved in sterile water and administered intraperitoneally at 80 mg/kg [15,37]. PG was suspended in 0.5% carboxymethylcellulose (CMC) and dosed orally at 30 mg/kg (low) or 60 mg/kg (high) [38]. The reference immunostimulant levamisole (LEV) was dissolved in water and given intraperitoneally at 2.5 mg/kg [39].

Experimental Design

The rats were distributed into five groups of five animals each (Table 1). Pre-treatment or vehicle was given for 14 days, and Groups II–V received CP during the final 7 days ([80mg/kg i.p]). Figure 1 outlines the timeline. On day 14, animals were anaesthetised with diethyl ether, blood was collected from the retro-orbital plexus, and the animals were euthanised so that the spleen and thymus could be harvested.

Table 1. Experimental groups.

Group	Designation	Treatment dose and route	Duration
I	Normal control	0.9% saline, 10 mL/kg	14 days
II	CP control (toxic)	Cyclophosphamide 80mg/kg,i.p	Last 7 days
III	Standard	Levamisole 2.5 mg/kg, i.p.	Last 14 days
IV	PG low dose	PG 30 mg/kg, p.o. (0.5% CMC)	Last 14 days
V	PG high dose	PG 60 mg/kg, p.o. (0.5% CMC)	Last 14 days

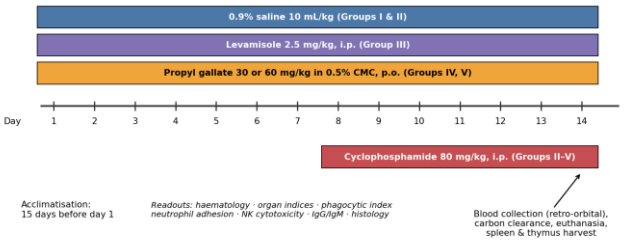

Figure 1. Study timeline. Test agents were given for 14 days; cyclophosphamide (CP) was administered during the final 7 days, and samples were collected on day 14. PG, propyl gallate.

Haematology

Red blood cell (RBC), white blood cell (WBC) and platelet counts were determined in anticoagulated blood using a Coulter LH755 haematology analyser [22].

Immune Organ Indices

The thymus and spleen were aseptically excised and weighed. For each organ, the index was obtained by dividing the group mean organ weight by the group mean body weight.

Macrophage Phagocytic Function (Carbon Clearance)

Macrophage phagocytic activity was assessed by the carbon clearance method [22]. Ink (10 mL/kg) was injected into a lateral tail vein, and 20 µL blood samples were withdrawn from the retro-orbital plexus at 2 min (t_1) and 10 min (t_2) and mixed with 2 mL of 0.1% Na_2CO_3. Absorbance at 600 nm was recorded (OD_1 and OD_2) and used to derive the phagocytic index.

Neutrophil adhesion and NK-cell cytotoxicity

Neutrophil adhesion (%) and splenic NK-cell cytotoxicity (%) were also determined.

Serum immunoglobulins

Blood was spun at 3500 rpm for 10 min at 4 °C, and serum IgG and IgM were measured using commercial ELISA kits following the manufacturer's instructions [22].

Histopathology

Spleen and thymus were fixed in 4% paraformaldehyde, paraffin-embedded and sectioned at 4 µm. After deparaffinisation, sections were stained with haematoxylin and eosin (H&E), dehydrated and viewed by light microscopy [13].

Statistical Analysis

Results are given as mean ± SEM. One-way analysis of variance followed by the Newman–Keuls multiple range test was used to compare groups, with $p < 0.01$ considered significant. In the tables, superscript a indicates a difference from the normal control and superscript b a difference from the CP control (both $p < 0.001$).tables, superscript a denotes a difference from the normal control and superscript b a difference from the CP control (both $p < 0.001$).

Results

Effect Of PG on Peripheral Blood Counts

CP caused marked pancytopenia (Table 2, Figure 2). Compared with the normal control, WBC, RBC and platelet counts dropped by 50.5%, 37.0% and 27.0%, respectively ($p < 0.001$). Both PG doses increased the WBC count (+57.9% at 30 mg/kg and +63.3% at 60 mg/kg relative to CP), reaching roughly 78% and 81% of control. Recovery of red cells and platelets depended clearly on dose: at 60 mg/kg the RBC and platelet counts exceeded those of the CP group by 22.6% and 20.3%, whereas at 30 mg/kg the RBC count (5.23) and platelet count (5.11) stayed near the CP values (5.57 and 4.98). Levamisole restored all three lineages to a greater extent than either PG dose.

Table 2. Effect of propyl gallate on blood counts in CP-treated rats.

Group	WBC (cells/µL)	RBC ($\times 10^6$/µL)	Platelets ($\times 10^5$/µL)
I – Normal control	7706.72 ± 36.99	8.84 ± 0.26	6.82 ± 0.13
II – CP control	3814.48 ± 32.52[a]	5.57 ± 0.14[a]	4.98 ± 0.06[a]
III – LEV 2.5 + CP	6374.79 ± 35.56[b]	7.23 ± 0.21[b]	6.41 ± 0.14[b]
IV – PG 30 + CP	6021.91 ± 19.22[b]	5.23 ± 0.55†	5.11 ± 0.33†
V – PG 60 + CP	6229.87 ± 27.17[b]	6.83 ± 0.15[b]	5.99 ± 0.07[b]

Mean ± SEM. a $p < 0.001$ versus normal control; b $p < 0.001$ versus CP control (one-way ANOVA, Newman–Keuls). † The source data flagged these values as significant, but they are close to the CP control; significance is withheld here pending verification against raw data. CP, cyclophosphamide; LEV, levamisole; PG, propyl gallate.

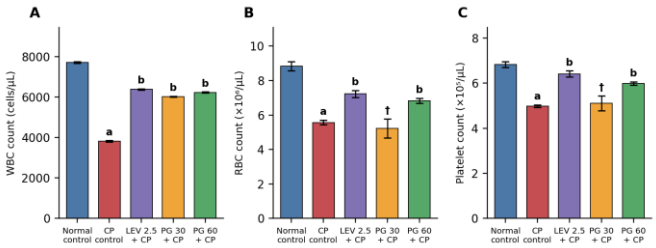

Figure 2. Blood counts after cyclophosphamide (CP) and propyl gallate (PG) treatment: (A) WBC, (B) RBC, (C) platelets. Bars are mean ± SEM. a, versus normal control; b, versus CP control; †, significance withheld (see Table 2).

Macrophage Phagocytosis and Neutrophil Adhesion

In the CP group the phagocytic index was 67.6% lower than in controls (0.012 versus 0.037; Table 3, Figure 3). PG raised it dose-dependently, to 0.036 at 30 mg/kg and 0.047 at 60 mg/kg; the higher value surpassed the normal control and approached that of levamisole (0.052). Neutrophil adhesion showed a similar trend: CP decreased it by 36.5%, and it increased to 37.8% and 46.8% following PG 30 and 60 mg/kg, respectively (levamisole, 52.7%).

Table 3. Effect of propyl gallate on carbon-clearance phagocytic index and neutrophil adhesion.

Group	Phagocytic index	Neutrophil adhesion (%)
I – Normal control	0.037 ± 0.001	32.09 ± 0.21
II – CP control	0.012 ± 0.002[a]	20.37 ± 0.69[a]
III – LEV 2.5 + CP	0.052 ± 0.002[b]	52.71 ± 0.86[b]
IV – PG 30 + CP	0.036 ± 0.002[b]	37.82 ± 0.34[b]
V – PG 60 + CP	0.047 ± 0.002[b]	46.83 ± 0.66[b]

Mean ± SEM. a $p < 0.001$ versus normal control; b $p < 0.001$ versus CP control.

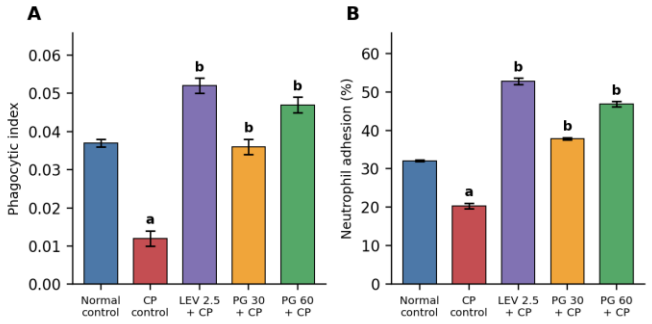

Figure 3. (A) Phagocytic index and (B) neutrophil adhesion (%). Bars are mean ± SEM; a, versus normal control; b, versus CP control.

Spleen and Thymus Indices

CP lowered the spleen index by 48.7% and the thymus index by 52.3% (Table 4, Figure 4). PG co-treatment restored both, with 60 mg/kg lifting the spleen index from 1.98 to 3.51 (+77.3%) and the thymus index from 0.83 to 1.42 (+71.1%). At the high dose, indices reached about 91% (spleen) and 82% (thymus) of control. The spleen showed the proportionally greater recovery.

Table 4. Effect of propyl gallate on spleen and thymus indices.

Group	Spleen index	Thymus index
I – Normal control	3.86 ± 0.03	1.74 ± 0.03
II – CP control	1.98 ± 0.05[a]	0.83 ± 0.03[a]
III – LEV 2.5 + CP	3.58 ± 0.02[b]	1.48 ± 0.02[b]
IV – PG 30 + CP	3.41 ± 0.01[b]	1.36 ± 0.01[b]
V – PG 60 + CP	3.51 ± 0.03[b]	1.42 ± 0.03[b]

Mean ± SEM. a $p < 0.001$ versus normal control; b $p < 0.001$ versus CP control.

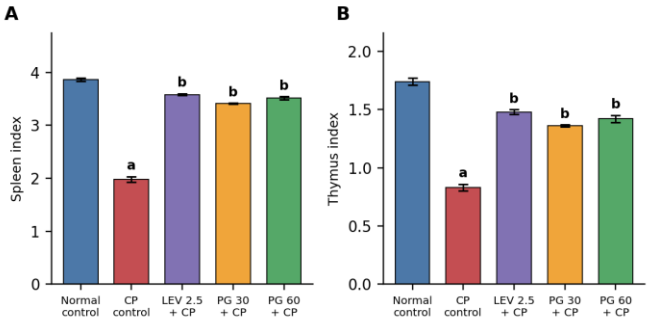

Figure 4. (A) Spleen index and (B) thymus index. Bars are mean ± SEM; a, versus normal control; b, versus CP control.

Splenic NK-cell cytotoxicity and Serum Immunoglobulins

NK-cell cytotoxicity fell from 93.8% in controls to 63.4% after CP (−32.5%; Table 5, Figure 5A). PG raised it to 79.2% (30 mg/kg) and 83.7% (60 mg/kg), against 86.8% for levamisole. CP reduced serum IgG and IgM by 43.6% and 54.7% (Table 5, Figure 5B–C). With PG at 30 and 60 mg/kg, IgG rose to 13.89 and 14.32 µg/mL and IgM to 7.93 and 8.20 µg/mL, equivalent to 84.6–87.3% (IgG) and 73.0–75.4% (IgM) of control values.

Table 5. Effect of propyl gallate on splenic NK-cell cytotoxicity and serum IgG and IgM.

Group	NK cytotoxicity (%)	IgG (µg/mL)	IgM (µg/mL)
I – Normal control	93.79 ± 1.11	16.41 ± 0.21	10.87 ± 0.13
II – CP control	63.35 ± 1.06[a]	9.26 ± 0.19[a]	4.92 ± 0.13[a]
III – LEV 2.5 + CP	86.81 ± 0.68[b]	14.97 ± 0.28[b]	8.66 ± 0.14[b]
IV – PG 30 + CP	79.24 ± 0.34[b]	13.89 ± 0.12[b]	7.93 ± 0.09[b]
V – PG 60 + CP	83.67 ± 0.92[b]	14.32 ± 0.16[b]	8.20 ± 0.10[b]

Mean ± SEM. a $p < 0.001$ versus normal control; b $p < 0.001$ versus CP control.

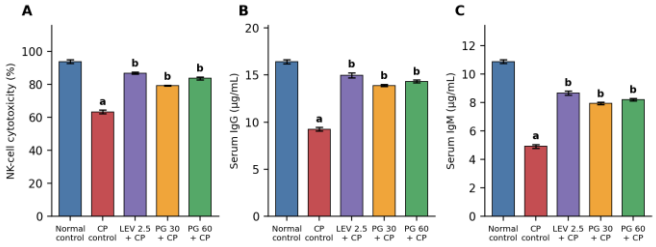

Figure 5. (A) Splenic NK-cell cytotoxicity, (B) serum IgG and (C) serum IgM. Bars are mean ± SEM; a, versus normal control; b, versus CP control.

Histology of Spleen and Thymus

Spleens from control animals contained densely packed lymphoid cells with well-stained nuclei and no sign of degeneration (Figure 6A). After CP, the spleen appeared irregular and sparsely cellular, with degenerating cells (Figure 6B). Levamisole and both PG doses yielded better-preserved cellularity, more uniform nuclei and less tissue disruption (Figure 6C–E), the 60 mg/kg dose appearing closest to normal. The thymus followed the same progression: dense, uniform lymphocytes in controls (Figure 7A), lowered cellularity with pale zones and partial loss of architecture after CP (Figure 7B), and gradually recovering lymphocyte density with levamisole or PG (Figure 7C–E). These observations are qualitative.degenerating cells (Figure 6B). Levamisole and both PG doses gave better-preserved cellularity, more uniform nuclei and less tissue disruption (Figure 6C–E), with the 60 mg/kg dose looking closest to normal. The thymus showed the same sequence: dense, uniform lymphocytes in controls (Figure 7A), reduced cellularity with pale areas and partial architectural loss after CP (Figure 7B), and progressively restored lymphocyte density after levamisole or PG (Figure 7C–E). These observations are qualitative.

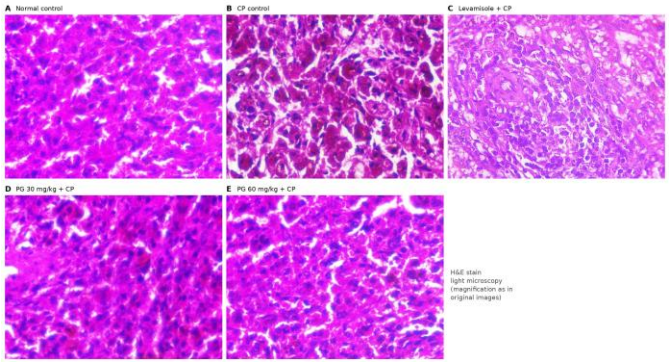

Figure 6. H&E-stained spleen sections: (A) normal control; (B) CP control; (C) levamisole + CP; (D) PG 30 mg/kg + CP; (E) PG 60 mg/kg + CP.

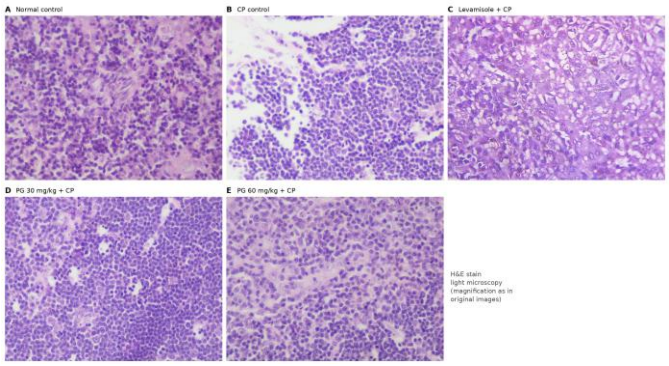

Figure 7. H&E-stained thymus sections: (A) normal control; (B) CP control; (C) levamisole + CP; (D) PG 30 mg/kg + CP; (E) PG 60 mg/kg + CP.

Integrated View of The Protective Effect

Table 6 and Figure 8 present all end-points on one scale. As the assays use different units, expressing each value relative to the normal control conveys both how deep the CP injury was and how far recovery went. With PG at 60 mg/kg most variables reached 75–91% of control (spleen index 91%, platelets 88%, IgG 87%, NK activity 89%), while the phagocytic index (127%) and neutrophil adhesion (146%) went above control values. The 30 mg/kg dose rescued leukocytes, organ indices, immunoglobulins and NK activity to a similar degree, but not RBC or platelets. Numerically, levamisole was the strongest agent for every parameter measured.

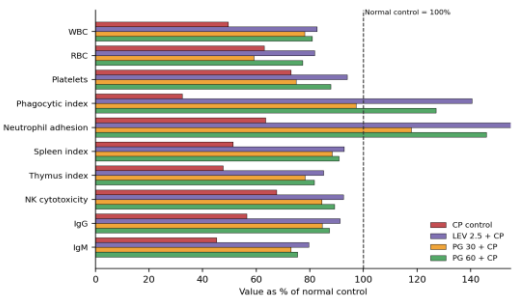

Figure 8. Recovery profile expressed as a percentage of the normal control for each end-point (group means from Tables 2–5). The dashed line marks 100% of the control value.

Table 6. Relative change produced by CP and by PG treatment (calculated from Tables 2–5).

Parameter	CP vs control (%)	LEV vs CP (%)	PG 30 vs CP (%)	PG 60 vs CP (%)
WBC	−50.5	+67.1	+57.9	+63.3
RBC	−37.0	+29.7	−6.1†	+22.6
Platelets	−27.0	+28.7	+2.6†	+20.3
Phagocytic index	−67.6	+333.3	+200.0	+291.7
Neutrophil adhesion	−36.5	+158.8	+85.7	+129.9
Spleen index	−48.7	+80.8	+72.2	+77.3
Thymus index	−52.3	+78.3	+63.9	+71.1
NK cytotoxicity	−32.5	+37.0	+25.1	+32.1
IgG	−43.6	+61.7	+50.0	+54.6
IgM	−54.7	+76.0	+61.2	+66.7

Percentage changes were calculated from group means. † Not distinguishable from the CP control (see Table 2).

Discussion

In this rat model, giving PG for two weeks together with a CP challenge reduced the decline in blood counts, maintained spleen and thymus weight and structure, and kept innate and humoral effector function nearer to normal. Most end-points responded in a dose-related fashion, and at 60 mg/kg the effects came close to those of the reference immunostimulant levamisole.function closer to normal. The effects were dose-related for most end-points and, at 60 mg/kg, approached those of the reference immunostimulant levamisole.

The pattern in blood counts reproduces the established myelosuppressive profile of CP: its active metabolite, phosphoramide mustard, alkylates the DNA of marrow progenitors, and the resulting loss of these cells lowers erythrocytes, leukocytes and platelets together [11,12,40,41]. At the higher dose PG lessened this effect across all three lineages, while at 30 mg/kg the benefit was limited to leukocytes, with RBC and platelet values close to those of the CP group. A possible explanation is that leukocyte progenitors are protected at lower exposure than erythroid and megakaryocytic precursors, though the present data cannot test this. Antioxidant interventions have improved blood indices in other models of chemotherapy toxicity [42,43], and reactive oxygen species participate in regulating erythropoietin production [44]. Whether PG acts by limiting oxidant damage to the marrow microenvironment is still to be demonstrated, since neither oxidative markers nor marrow were examined.

Spleen and thymus indices declined by about half after CP, consistent with the lymphoid depletion described in other

CP models [13,16,18,22]. Both PG doses restored these indices, and the histology matched the weights: CP-treated tissues were hypocellular and disrupted, while PG-treated tissues kept dense lymphoid parenchyma. The thymus is where T cells mature and the spleen is the principal peripheral site for filtering antigen and expanding lymphocytes, so sparing these organs is a meaningful correlate of retained immune capacity. Other immunostimulatory agents have likewise been reported to restore organ mass [45,46].

Innate effector function likewise improved. CP depressed carbon clearance and neutrophil adhesion, and PG returned both to, or beyond, control levels. NK cells take part in early antiviral and antitumour defence and in regulating cytotoxic T-cell responses [47], and diminished NK activity is a sensitive marker of CP immunotoxicity [48]. Here, PG at 60 mg/kg increased NK cytotoxicity from 63.4% to 83.7%, compared with 86.8% for levamisole. Since phagocytosis, neutrophil adhesion and NK function are all vulnerable to oxidative injury, a common upstream effect of PG on redox balance is a reasonable hypothesis (Figure 9), although this study does not establish it.

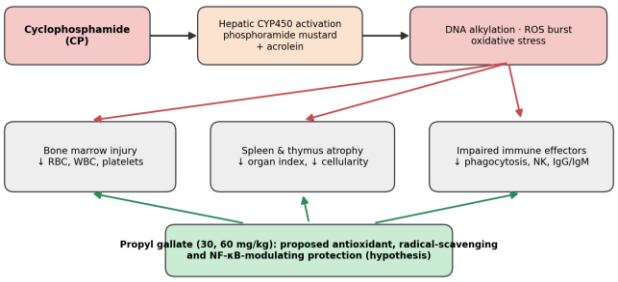

Figure 9. Proposed (hypothetical) scheme. CP is bioactivated to phosphoramide mustard and acrolein, causing DNA alkylation and oxidative stress that damage marrow, lymphoid organs and immune effector cells. PG may counteract these events through antioxidant and NF-κB-modulating actions; this pathway was not tested directly here.

IgG and IgM fell by 44–55% after CP, in keeping with reduced B-cell and plasma-cell output, and PG substantially restored both. IgM predominates in the primary response, whereas IgG mediates most of the secondary and long-term response [4]; recovery of both classes therefore suggests broadly preserved humoral competence. The higher levels may reflect protection of splenic lymphoid tissue rather than direct B-cell stimulation, and an immunisation challenge would be needed to distinguish the two.

The mechanisms proposed for PG come from other experimental systems. PG blocks NF-κB activation and COX-2 expression in monocytes [27], diminishes ROS-dependent NF-κB signalling in glioma cells [31], and protects against cardiac and hepatic injury in models of those conditions [30,34]. Conversely, it induces ROS and autophagy in hepatocellular carcinoma cells [35] and alters glutathione and caspase-dependent signalling in lung cancer cells [36]. This dual behaviour matters: before PG could be used with CP in cancer patients, it would have to be confirmed that PG does not weaken the antitumour activity of CP.

As comparator, levamisole was numerically better than PG for most measures, as expected of an established immunostimulant, yet PG at 60 mg/kg came within a modest margin of it for leukocyte count, organ indices, NK activity and immunoglobulins (Table 6). The two agents probably act by different routes: levamisole is a recognised immune adjuvant, whereas PG is chiefly an antioxidant, and this distinction could be clinically relevant because a protective antioxidant would be expected to preserve immune function rather than overstimulate it. PG lifted phagocytosis and neutrophil adhesion above control, showing that these end-points respond to stimulation as well as to restoration.

Numerous botanical extracts, polysaccharides and purified compounds are reported to reduce CP-induced haematological and immune toxicity [15,17,20,26]. PG stands apart as a defined, low-molecular-weight compound of known structure, so dosing and mechanistic investigation are easier to control than with crude preparations. Doses were selected from earlier rodent studies with PG [38]; a broader dose–response study, along with pharmacokinetic data in CP-treated animals, would clarify the exposure required for protection.

Several limitations apply. The groups were small and no PG-only control was included, so the intrinsic effects and safety of PG in animals that are not immunosuppressed remain unknown. Only two PG doses and a single CP regimen were tested, and the design lacked oxidative-stress markers (for example MDA, GSH, SOD), cytokine or Th1/Th2 profiling [49,50], bone-marrow histology and a functional immunisation challenge. Histology was evaluated qualitatively, without scoring. Lastly, healthy rats rather than tumour-bearing animals were used, so nothing can be concluded about any effect on the antitumour efficacy of CP.

Conclusion

Propyl gallate, most notably at 60 mg/kg, opposed CP-induced immunosuppression in Wistar rats: it improved blood counts, maintained spleen and thymus indices and architecture, and restored phagocytic activity, neutrophil adhesion, NK-cell cytotoxicity and serum IgG and IgM. Redox-related mechanisms are plausible but unproven. Before any translational claim is made, future studies should add a PG-only group, oxidative and cytokine

profiling, marrow assessment and tumour-bearing models.

Abbreviations

CMC, carboxymethylcellulose; CP, cyclophosphamide; ELISA, enzyme-linked immunosorbent assay; GSH, reduced glutathione; H&E, haematoxylin and eosin; IAEC, Institutional Animal Ethics Committee; Ig, immunoglobulin; LEV, levamisole; MDA, malondialdehyde; NK, natural killer; PG, propyl gallate; RBC, red blood cell; ROS, reactive oxygen species; SEM, standard error of the mean; SOD, superoxide dismutase; WBC, white blood cell.

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