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Original article

Haematological Alterations Induced by Valproic Acid and the Protective Role of Nano Formulation in Male Rabbits

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Abstract
Valproic acid (VPA) is a widely prescribed antiepileptic drug whose long-term use is frequently associated with haematological toxicity. The present study aimed to evaluate the haematological alterations induced by valproic acid and to investigate the protective potential of a synthesised nano-co-polymer formulation in male rabbits. The nano-co-polymer was prepared via an esterification process and structurally characterised using FT-IR and ^1H-NMR spectroscopy, which confirmed successful ester bond formation and aromatic substitutions. Twenty male rabbits were randomly allocated into four groups (n = 5): a control group, a VPA-treated group (200 mg/kg), a nano-formulation group (100 mg/kg), and a combined VPA + nano-formulation group, treated for six weeks. Haematological parameters, including haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), hematocrit (HCT), platelets (PLT), and red cell indices (MCV, MCH, MCHC), were assessed using an automated haematology analyser. VPA administration significantly reduced haemoglobin concentration (11.5 ± 0.23 g/dL) compared with the control group (13.4 ± 0.28 g/dL, $P < 0.05$), accompanied by a significant decrease in WBC count (7.9 ± 0.31 $\times 10^3$/µL versus 8.83 ± 0.26 $\times 10^3$/µL). A concomitant reduction in hematocrit was also observed (37.33 ± 0.71 vs. 40.27 ± 0.25), indicating mild anaemia and immunosuppression. In contrast, rabbits treated with the nano formulation alone showed haematological values comparable to, or slightly higher than, controls, particularly WBC count (9.4 ± 0.39 $\times 10^3$/µL). Notably, co-administration of the nano formulation with VPA significantly ameliorated VPA-induced haematological disturbances, restoring Hb, WBC, and HCT levels toward normal ranges. No significant alterations were observed in RBC count, platelet count, or red cell indices among the experimental groups. In conclusion, valproic acid induces mild but significant haematological toxicity in male rabbits, mainly affecting haemoglobin and leukocyte levels. The synthesised nano-co-polymer formulation demonstrated a clear protective effect, attenuating VPA-induced haematological alterations. These findings highlight the potential of nano-based formulations as supportive therapeutic strategies to reduce drug-induced hematotoxicity and enhance treatment safety.
Keywords. Valproic Acid; Nano Formulation; Haematological Toxicity; Nanopolymers; Male Rabbits.

Introduction

Although valproic acid is a commonly used antiepileptic and mood-stabilising medication, its haematological toxicity frequently limits its therapeutic advantages [1]. According to several studies, long-term exposure to valproic acid can cause oxidative stress and direct disruption of hematopoietic cell proliferation, which can result in bone marrow suppression, decreased erythropoiesis, anaemia, thrombocytopenia, and leukocyte abnormalities [2,3]. These changes are believed to be the consequence of both compromised antioxidant defence systems and increased production of reactive oxygen species, which together cause cellular damage in blood-forming tissues [4].

Formulations based on nanotechnology have recently drawn interest as potential defence mechanisms against drug-induced toxicity. In addition to improving bioavailability and the pharmacokinetic behaviour of medicinal drugs, nanoparticles have inherent anti-inflammatory and antioxidant properties [5-7]. Because of their tiny size, they may more effectively penetrate cells and engage with oxidative pathways, which may lessen tissue damage and restore haematological balance. Haematological and biochemical changes brought on by hazardous chemicals can be lessened by nano-formulated antioxidants and natural compounds, according to experimental research [8-10].

Innovative drug-delivery technologies including biosensors and tailored nanocarriers are made possible by nanotechnology [11,12]. By lowering particle size without compromising drug-loading capacity, researchers hope to access deeper cellular compartments, particularly when focusing on delicate areas like the central nervous system. For use in biomedical applications, a variety of organic and inorganic nanomaterials with enhanced structural, chemical, mechanical, and biological properties have been created [13-17].

Methods

Synthesis of co-polymer nanoparticle

Esterification is the process utilised to synthesise the nano-co-polymers; the preparation procedure is explained below: Phthalic anhydride (1.5 moles, 222 g) and "DMSO" (33 mL) were combined in a 200 mL beaker. There was a thermometer in this beaker. Using a hot plate magnetic stirrer, the mixture was gently heated to 70°C until a clear liquor was formed. The solution was then supplemented with 1.0 mole, 92 g of glycerol. After the mixture was carefully heated to 100 degrees Celsius, 10 millilitres of p-xylene were added to the reaction beaker in batches of three drops each; the water created by the esterification process was removed, and the beaker was gently heated. In order to produce the nano-copolymer, heating

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was halted after 52 minutes at 117 degrees Celsius till no more water came out. The suspension solution was then created by adding the cold distilled water. After letting the suspension solution precipitate for the whole night, it was filtered, washed with distilled water, and allowed to dry. FT-IR and 1HNMR methods were used to characterise the produced nano-co-polymer. The manufacture of the nano-co-polymer is depicted in (Figure 1).

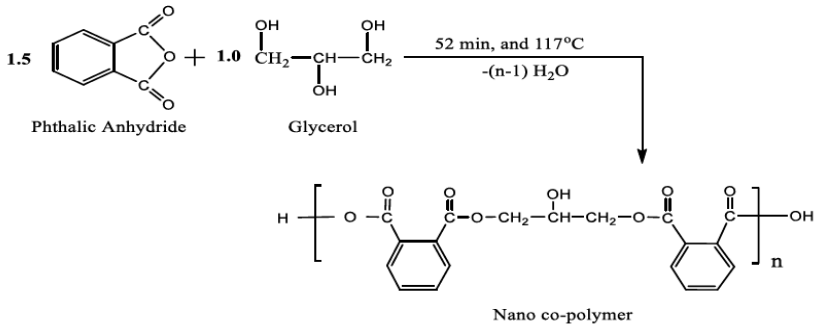


Figure 1. Manufacture of the nano-co-polymer

Haematological effects of VPA

Twenty healthy male rabbits were obtained from reputable local farms and housed in stainless-steel cages under conditions compliant with US-EPA (2004) and Libyan Ministry of Agriculture guidelines, with a 12-hour light/dark cycle, temperature of 22–26°C, and relative humidity of 40–70%, having free access to clean water and a balanced diet throughout the study. Rabbits were randomly divided into four groups (n = 5 per group) and treated as follows: Group 1 received valproic acid (200 mg/kg body weight) orally on alternate days for six weeks, Group 2 received a nano formulation (100 mg/kg body weight) orally daily for six consecutive weeks, Group 3 received a combined treatment of valproic acid (200 mg/kg) and the nano formulation (100 mg/kg) daily for six weeks, and Group 4 served as the control, receiving 8 ml of distilled water orally for six weeks[18]. Weekly body weights and clinical observations were recorded, and blood samples were collected from the ear vein for complete blood count (CBC) analysis, including haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), hematocrit (HCT), platelets (PLT), and red cell indices (MCV, MCH, MCHC), using the XP-300 Automated Haematology Analyser (Sysmex, USA) with manual differential counts performed on Dif-Quik-stained smears. At the end of the experiment, rabbits were euthanised 24 hours after the last treatment, and blood was collected by cardiac puncture, divided into heparinised tubes for haematological assays. Statistical analysis was performed using Minitab 17 or GraphPad Prism 8, with normality assessed before applying one-way ANOVA followed by Tukey's multiple comparison test, considering $P < 0.05$ as statistically significant, and graphical representations were prepared using Microsoft Excel 2007 with logarithmic scales applied where appropriate.

Results and Discussion

The FT-IR spectrum of the synthesised nano-co-polymer (Figure 2) provides clear evidence for successful esterification and polymer formation. The appearance of a weak, broad absorption band around 3300 cm^{-1} is attributed to O–H stretching vibrations of alcoholic groups and intermolecular hydrogen bonding, indicating the presence of residual hydroxyl functionalities within the polymeric structure. The stretching band observed at 3003 cm^{-1} corresponds to aromatic C–H vibrations, confirming the incorporation of aromatic moieties into the co-polymer backbone. A strong and characteristic absorption band at 1669 cm^{-1} is assigned to the ester carbonyl (C=O) group, which represents a key indicator of ester bond formation during the synthesis process. Additionally, the peak at 1069 cm^{-1} is attributed to C–O stretching vibrations of the ester linkage, further supporting successful esterification. The bands observed at 734 and 897 cm^{-1} are characteristic of di-substituted aromatic rings, confirming the structural integrity of the aromatic system within the nano co-polymer.

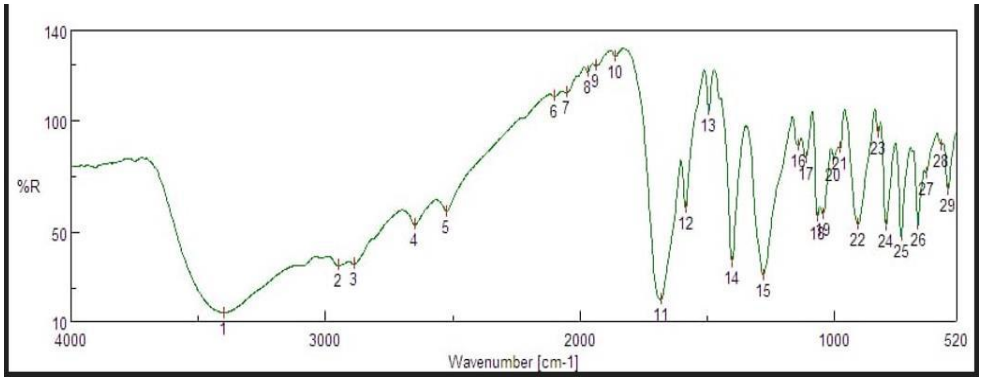


Figure 2. The FT- IR spectrum of the nano-copolymer compound

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The structural features identified by FT-IR analysis are further supported by the ^1H-NMR spectrum (Figure 3), which provides complementary evidence for nano-copolymer formation. The appearance of a singlet signal at approximately 13 ppm is assigned to the acidic O–H proton, indicating the presence of residual carboxylic or hydroxyl groups. The doublet signal observed around 8 ppm corresponds to aromatic protons (Ph–H), confirming the aromatic environment within the polymer matrix. Furthermore, the signal detected at approximately 2.5 ppm is attributed to protons associated with ester-related functional groups, supporting the formation of ester linkages within the polymer structure. Overall, the combined FT-IR and ^1H-NMR analyses confirm the successful synthesis of the nano-copolymer with well-defined ester bonds and aromatic substitutions. These spectral features are in good agreement with previously reported polymeric nanomaterials synthesised via esterification reactions, reinforcing the structural validity and chemical stability of the prepared nano formulation [19-21].

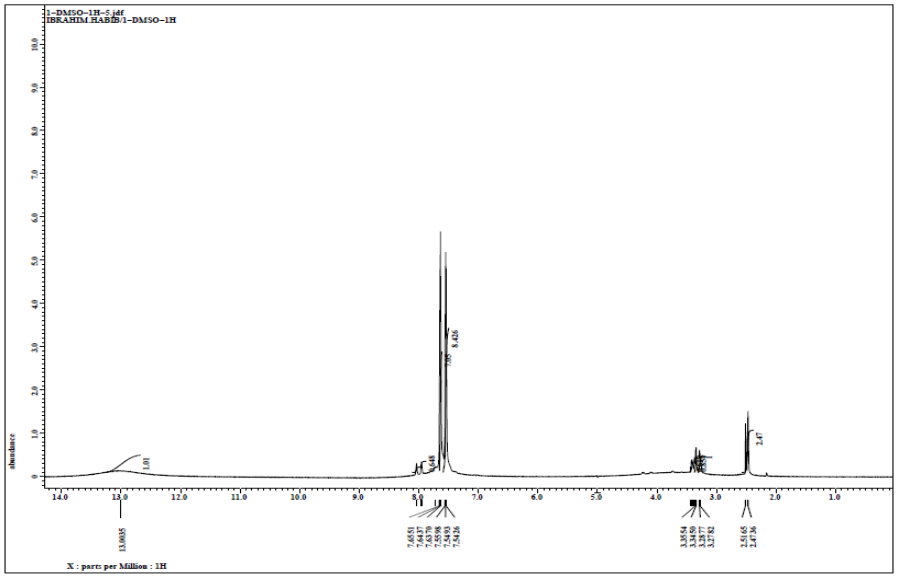


Figure 3. The 1HNMR spectrum of nano-co-polymer compound

Investigated the haematological and hormonal effects of VPA and the protective role of a nano formulation in male rabbits. The data presented in Table 1 demonstrate the impact of valproic acid, the nano formulation, and their combination on the complete blood count (CBC) of male rabbits. Treatment with valproic acid alone resulted in a significant decrease in haemoglobin (Hb) concentration (11.5 ± 0.23 g/dl) compared with the control group (13.4 ± 0.28 g/dl), suggesting a mild anaemic effect likely due to oxidative damage to erythrocytes or bone marrow suppression caused by the drug. In contrast, the nano-treated and combined groups exhibited Hb values statistically comparable to the control, indicating a potential ameliorative or protective effect of the nano preparation against valproic acid–induced hematotoxicity. Although red blood cell (RBC) counts did not differ significantly among groups, the reduction in hematocrit (HCT) observed in the valproic acid group (37.33 ± 0.709) compared with the control (40.27 ± 0.250) further supports the mild anaemia indicated by the lower Hb level. White blood cell (WBC) counts showed a significant decrease in the valproic acid group (7.9 ± 0.31 × 10^3/µl), which may reflect immunosuppression or leukocyte toxicity associated with the drug.

Conversely, the nano group had the highest WBC count (9.4 ± 0.39 × 10^3/µl), suggesting enhanced immune response or restoration of leukopoiesis. Platelet (PLT) counts and red cell indices (MCV, MCH, and MCHC) did not show statistically significant changes across groups, indicating that the structural integrity of red blood cells was largely maintained. The near-normal values in the combination group further imply that the nano formulation may mitigate the adverse haematological alterations induced by valproic acid. Overall, these findings indicate that valproic acid exerts mild haematological toxicity manifested mainly by decreased Hb and WBC levels, while the nano compound, either alone or combined with valproic acid, shows a protective role in maintaining normal blood parameters in male rabbits.

The haematological findings of the present study demonstrate that valproic acid exerts measurable toxic effects on blood parameters in male rabbits, particularly reflected in the significant reduction in haemoglobin and WBC counts. The decrease in Hb observed in the valproic acid group supports earlier reports indicating that valproic acid can induce anaemia through oxidative injury to erythrocytes or suppression of bone marrow activity [1,22,23]. Several studies have shown that VPA increases the production of reactive oxygen species, which may accelerate red blood cell destruction or impair erythropoiesis, ultimately leading to reduced haemoglobin concentration and hematocrit levels. This aligns well with the lower HCT recorded in the VPA-treated rabbits, suggesting mild anaemia consistent with drug-induced hematotoxicity [7,24]. The marked reduction in WBC count following valproic acid administration further highlights its immunosuppressive potential. This effect has been previously associated with leukocyte toxicity or inhibition of leukocyte proliferation through oxidative stress mechanisms. The decline in WBCs may weaken immune function and increase susceptibility to infections, a known side effect of chronic VPA therapy [5].In contrast, rabbits treated with the nano formulation exhibited a significant elevation in WBC count compared with both the control and VPA groups. This suggests that the nano compound may stimulate immune activity or counteract oxidative suppression of leukopoiesis, potentially through its antioxidant or anti-

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inflammatory properties [25]. Notably, the combination treatment (valproic acid plus nano formulation) resulted in intermediate Hb and WBC values, indicating partial protection against VPA-induced haematological alterations [26-29,35-38]. This improvement suggests that the nano formulation may mitigate hematotoxic effects by scavenging free radicals, stabilising cell membranes, or supporting bone marrow function.

The normalisation seen in Hb and WBC values in the combined group reinforces the idea that nano-based agents may serve as effective co-therapeutic options when managing drugs associated with haematological toxicity [3,4]. Other haematological indices, including RBC count, platelet count, and red cell indices (MCV, MCH, MCHC), remained largely unchanged across all groups. This stability suggests that while valproic acid predominantly affects haemoglobin synthesis and leukocyte counts, it may not significantly alter red cell morphology or platelet production at the administered dose and duration. The near-normal values observed in the nano-treated and combination groups further emphasise the biocompatibility and protective nature of the nano formulation [1,23,30-34].

Table 1. Changes in red blood cells (RBCs), white blood cells (WBCs), haemoglobin (Hb", packed cell volume "PCV", platelets, and hemoglobin (Hb) were all included in the total blood counts of male rabbits treated with valproic acid, nano, and their combination.

Parameter	Groups			
	Control	Valproic acid	Nano	Valproic acid +Nano
Hb (g/dl)	13.4 ±0.28 g/dL	11.5 ± 0.23 g/dL	13.25 ± 0.33 g/dL	12.77 ± 0.21 g/dL
RBC $\times 10^6$ (µl)	5.59 ± 0.386 µl	5.37 ± 0.092 µl	5.78 ± 0.55 µl	5.58 ± 0.148 µl
WBC $\times 10^3$(µl)	8.83±0.26 µl	7.9 ± 0.31 µl	9.4 ± 0.39 µl	8.5 ± 0.44 µl
PLAT $\times 10^3$(µl)	270.73 ± 13.85 µl	291.93± 12.12 µl	255.34 ± 24.49 µl	271.0 ± 17.59 µl
HCT$\times 10^3$(µl)	40.27 ± 0.250%	37.33 ± 0.709%	37.79 ± 1.500%	40.18± 1.230%
MCV$\times 10^3$(µl)	77.11 ± 2.46fL	77.84 ± 1.37fL	77.48 ± 1.73fL	76.57± 2.67fL
MCH$\times 10^3$(µl)	25.32 ±2.35pg	28.40 ±1.58pg	24.6±1.31pg	25.6±1.96pg
MCHC$\times 10^3$(µl)	32.59±0.921g/dL	32.46±0.446g/dL	33.93±1.05g/dL	32.25±0.277g/dL

Values are expressed as mean ± standard error (SE). Different superscript letters within the same row indicate statistically significant differences among groups at P < 0.05.

Conclusion

Valproic acid induced mild but significant haematological toxicity in male rabbits, mainly reflected by reduced haemoglobin, white blood cell count, and hematocrit. The synthesised nano-co-polymer was structurally confirmed and showed good biocompatibility without adverse haematological effects. Co-administration of the nano formulation effectively mitigated valproic acid–induced alterations, highlighting its potential as a supportive strategy to reduce drug-related hematotoxicity.

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