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TOXICOLOGICAL EVALUATION OF PURE POTASSIUM SORBATE AND COMMERCIAL FORMULATIONS: ACUTE AND SUBCHRONIC TOXICITY, GENOTOXICITY, AND BIOCHEMICAL PARAMETERS IN RODENTS

AVALIAÇÃO TOXICOLÓGICA DO SORBATO DE POTÁSSIO PURO E DE FORMULAÇÕES COMERCIAIS: TOXICIDADE AGUDA E SUBCRÔNICA, GENOTOXICIDADE E PARÂMETROS BIOQUÍMICOS EM ROEDORES

EVALUACIÓN TOXICOLÓGICA DEL SORBATO DE POTASIO PURO Y DE FORMULACIONES COMERCIALES: TOXICIDAD AGUDA Y SUBCRÓNICA, GENOTOXICIDAD Y PARÁMETROS BIOQUÍMICOS EN ROEDORES

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ABSTRACT: This study evaluated the acute and subchronic toxicity of pure potassium sorbate and investigated the genotoxic, hematological, biochemical, and histopathological effects of commercial formulations based on this preservative in rodents. The toxicity of pure potassium sorbate was assessed following OECD 425 and 407 guidelines. The commercial formulations Golden Blue, Aquafiv, and Aquativagro were evaluated through comet assays (in L929 cells) and micronucleus tests, alongside hematological, serum biochemical, and histopathological examinations of the liver and kidneys. No mortality or significant body weight alterations were observed with pure potassium sorbate, indicating an oral LD50 above 1,100 mg/kg. Commercial formulations showed no genotoxic effects. However, significant reductions in erythrocyte, total leukocyte, and monocyte counts were observed, accompanied by decreased serum ALT and AST activities and increased creatinine concentrations. Histopathological analyses revealed glomerular congestion, tubular swelling, vacuolar degeneration, hepatic vascular congestion, and microvesicular steatosis. In conclusion, pure potassium sorbate exhibited low acute toxicity, while commercial formulations induced distinct hepatorenal and hematological alterations, highlighting the need for further safety assessments.

Keywords: Genotoxicity. Histopathology. Food Preservatives.

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RESUMO: Este estudo avaliou a toxicidade aguda e subcrônica do sorbato de potássio puro e investigou os efeitos genotóxicos, hematológicos, bioquímicos e histopatológicos de formulações comerciais à base desse conservante em roedores. A toxicidade do composto puro seguiu as diretrizes OECD 425 e 407. As formulações Golden Blue, Aquafiv e Aquativagro foram submetidas aos ensaios cometa (células L929) e micronúcleo, além de análises hematológicas, bioquímicas e histopatológicas de rins e fígado. Não houve mortalidade ou alterações no ganho de peso corporal com sorbato de potássio puro, indicando DL50 oral superior a 1.100 mg/kg. As formulações comerciais não induziram genotoxicidade nos ensaios cometa e de micronúcleo. Contudo, observou-se redução na contagem de hemácias, leucócitos totais e monócitos, associada à diminuição das atividades de ALT e AST e ao aumento da creatinina sérica. A análise histopatológica evidenciou congestão glomerular, tumefação do epitélio tubular cortical, degeneração vacuolar, congestão vascular hepática e esteatose microvesicular. Conclui-se que o sorbato de potássio puro exibe baixa toxicidade aguda, mas as formulações comerciais promovem alterações hepatorrenais e hematológicas, demandando cautela em seu uso continuado.

Palavras-chave: Genotoxicidade. Histopatologia. Conservantes alimentares.

RESUMEN: Este estudio evaluó la toxicidad aguda y subcrónica del sorbato de potasio puro e investigó los efectos genotóxicos, hematológicos, bioquímicos e histopatológicos de formulaciones comerciales a base de este conservante en roedores. La toxicidad del compuesto puro se evaluó según las directrices OECD 425 y 407. Las formulaciones Golden Blue, Aquafiv y Aquativagro se sometieron al ensayo cometa (en células L929) y al test de micronúcleos, además de análisis hematológicos, bioquímicos e histopatológicos de hígado y riñones. No se observó mortalidad ni alteraciones en el peso corporal con el sorbato de potasio puro, determinando una DL50 oral superior a 1.100 mg/kg. Las formulaciones comerciales no demostraron genotoxicidad. Sin embargo, se registraron reducciones en el recuento de eritrocitos, leucocitos totales y monocitos, junto con una disminución de ALT y AST y un aumento de creatinina sérica. El examen histopatológico reveló congestión glomerular, tumefacción tubular, degeneración vacuolar y esteatosis microvesicular hepática. En conclusión, el sorbato de potasio puro presentó baja toxicidad aguda, mientras que las formulaciones comerciales generaron alteraciones hepatorrenales y hematológicas relevantes.

Palabras clave: Genotoxicidad. Histopatología. Conservantes alimentarios.

INTRODUCTION

Potassium sorbate is widely employed as an antifungal additive and chemical preservative in industrial food, pharmaceutical, and animal feed products due to its efficacy in preventing yeast and mold spoilage. Although recognized as Generally Recognized as Safe (GRAS) by major international regulatory agencies within established limits, rigorous toxicological screening remains imperative to prevent potential risks associated with prolonged dietary intake or high cumulative exposures (Butterworth et al., 1975; American College of Toxicology, 1988). Contemporary toxicological investigations have raised questions regarding continuous additive exposure, highlighting potential mechanisms involving metabolic

disruption, hepatorenal stress, and cellular oxidative damage (Abd-Elhakim et al., 2023; Pinto et al., 2025; Zhang et al., 2025).

In this scenario, acute and subchronic repeated-dose toxicity studies represent standardized steps to delineate primary lethal doses and systemic physiological responses according to guidelines established by the Organisation for Economic Co-operation and Development (OECD, 2008a; OECD, 2008b). Recent evidence suggests that extended exposure to potassium sorbate can stimulate inflammatory cascades and oxidative stress in hepatic and renal parenchymas, primarily mediated by the TLR2/TLR4/NF-κB signaling pathways (Abd-Elhakim et al., 2023). Furthermore, animal model studies indicate that continuous intake may perturb gut microbiota composition, contributing to metabolic imbalance and abnormal lipid deposition (Zhang et al., 2025).

Genotoxicity assessment is an equally critical component of chemical safety profiling to prevent DNA damage that can lead to genomic instability or neoplastic transformations (Santos et al., 2020). Standardized methodologies, such as the alkaline single-cell gel electrophoresis (comet assay) and the micronucleus test, serve as reliable biomarkers to detect DNA single/double-strand breaks, crosslinks, and chromosomal structural aberrations (Fenech, 2000; Hayashi, 2016). While systematic reviews report variable genotoxic responses to food preservatives across experimental models (Pinto et al., 2025), computational toxicological workflows and molecular docking have also identified prospective interactions between potassium sorbate and key cellular targets, such as MMP9, APP, and TLR4 (Wu et al., 2025).

Systemic toxicity can be sensitively detected through hematological and biochemical panels. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, blood urea nitrogen, creatinine, and glucose provide validated insights into hepatocellular integrity, renal excretory function, and glucose homeostasis (Kaneko et al., 2008; Latimer, 2011; Thrall et al., 2012). Previous studies evaluating 90-day subchronic exposures in rodents noted marked elevations in transaminases and nitrogenous wastes coupled with compromised antioxidant defenses (Abd-Elhakim et al., 2023).

Therefore, this study aimed to determine the acute and subchronic oral toxicity of pure potassium sorbate in rodents and to evaluate the genotoxic, hematological, biochemical, and histopathological effects of three commercial preservative formulations (Golden Blue, Aquafiv, and Aquativagro). Evaluating fully formulated commercial products accounts for the potential synergistic or additive effects of industrial excipients and adjuvants, providing a more

realistic assessment of occupational, domestic, or dietary exposure scenarios (Dearfield et al., 2002; Kirkland et al., 2007).

METHODS

Ethical considerations

All experimental animal protocols were conducted in strict accordance with national animal welfare regulations and were formally reviewed and approved by the Ethics Committee on Animal Use (CEUA) of the State University of Ceará (UECE), under protocol number 2292169/2018.

Acute oral toxicity

Acute toxicity was evaluated using 32 female Swiss mice (*Mus musculus*), weighing 21 ± 4 g, acclimatized for 15 days in polypropylene cages under controlled photoperiod conditions (12 h light/12 h dark cycle) and ambient temperature (22 ± 1 °C). Animals received commercial rodent chow (Labina®, Purina, São Paulo, Brazil) and filtered water *ad libitum*. Testing was performed using the Up-and-Down Procedure in accordance with OECD Test Guideline 425 (OECD, 2008b). Animals received a single oral gavage of pure potassium sorbate in a fixed volume of 0.3 mL, sequentially testing doses of 175, 440, and 1,100 mg/kg. The progression of dosing was determined by the survival or toxicity status of the preceding animal. A naive control group was maintained under identical conditions for comparison. Animals were monitored continuously for clinical signs of toxicity during the first 48 hours and followed for 14 days, with individual body weight recordings. Median lethal dose (LD50) estimation was carried out using AOT425StatPgm software (OECD, 2008b).

Subchronic oral toxicity

Subchronic evaluation was performed on 32 female Wistar rats (*Rattus norvegicus*), weighing 250 ± 15 g, acclimatized for 30 days under standard laboratory conditions. The protocol was adapted from OECD Test Guideline 407 (OECD, 2008a) for a 30-day continuous oral exposure period. The animals were randomly assigned to four experimental cohorts (n = 8/group): an untreated control group and three treatment groups receiving daily oral doses of pure potassium sorbate at 175, 440, or 1,100 mg/kg (0.3 mL volume). Clinical manifestations,

behavioral changes, and mortality were logged daily. Individual body weights were recorded on days 0, 15, and 30 for dosage adjustment and growth monitoring.

Genotoxicity assessment

In vitro alkaline comet assay

The comet assay was conducted using the murine fibroblast cell line L929 according to the protocol by Singh et al. (1988), with technical adaptations described by Hartmann and Speit (1997) following international guidelines (Tice et al., 2000). Cells were treated with commercial formulations containing potassium sorbate (Golden Blue, Aquafiv, and Aquativagro) at dilutions of 1:1000, 1:500, and 1:100. Methyl methanesulfonate (MMS) was used as the positive control. Following detachment with 0.15% trypsin, cells were resuspended in complete RPMI medium, embedded in low-melting-point agarose on precoated slides, and immersed in cold lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, 10% DMSO, pH 10.0) at 4 °C for at least 1 hour. To evaluate potential DNA-protein crosslinks, an enzymatic digestion step was performed on duplicate slide sets using Proteinase K (200 μg/mL in TE buffer, pH 8.0) for 60 minutes at 37 °C (Cavalcanti et al., 2006; Collins et al., 2023). Alkaline electrophoresis was run for 20 minutes at 25 V and 300 mA (0.86 V/cm). One hundred randomly selected nucleoids per group were scored under fluorescence microscopy to calculate the DNA damage index (ranging from 0 to 400 arbitrary units) and damage frequency (Cavalcanti et al., 2006).

In vivo micronucleus assay

The micronucleus assay was performed on peripheral blood smears obtained from rodents exposed to the three commercial formulations (1:1000, 1:500, and 1:100 dilutions) and positive controls, as described by Fenech M (2000). Blood smears were air-dried overnight, fixed in pure methanol for 15 minutes, stained with 5% Giemsa solution for 40 minutes, and examined under high-power optical microscopy following standardized scoring criteria (Fenech et al., 2003).

Hematological and serum biochemical analyses

Animals from the in vivo cohort exposed to the highest concentration of the commercial formulations (1:100 dilution; n = 6/group) and the control group were evaluated after 30 days of

treatment. Blood samples were collected under deep anesthesia induced with ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (10 mg/kg) (Fish et al., 2008).

Whole blood samples (3 mL) collected into K2-EDTA tubes were analyzed to determine red blood cell count (RBC), hemoglobin concentration (HGB), total leukocyte count, lymphocyte count, and monocyte count, following standard laboratory methods (Thrall et al., 2012).

Serum obtained from non-anticoagulated tubes after centrifugation was analyzed for renal markers (urea and creatinine) (Kaneko et al., 2008), liver markers (ALT, AST, total bilirubin, and direct bilirubin) (Thrall et al., 2012), and serum glucose levels (Latimer, 2011). All analyses were executed on certified spectrophotometric platforms using commercial diagnostic kits (LABTEST® Diagnóstica, Brazil).

Histopathological processing

Following blood collection, the animals were euthanized via anesthetic overdose (xylazine 30 mg/kg + ketamine 240 mg/kg). Liver and kidney samples were carefully dissected and fixed in 10% neutral buffered formalin for 48 hours. The tissues were dehydrated in graded ethanol series, cleared in xylene, and embedded in paraffin wax. Tissue sections (5 μm) were stained with Hematoxylin and Eosin (H&E) for light microscopy examination. Morphological alterations were semi-quantitatively scored following criteria adapted from Abd-Elhakim et al. (2023): absent (–), mild (+), moderate (++), and severe (+++).

Statistical analysis

Data normality was verified using the Shapiro-Wilk test, and values were expressed as mean ± standard deviation (SD). Parametric datasets were evaluated by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. Non-parametric datasets were analyzed using the Kruskal-Wallis test followed by Dunn's post-hoc test. Statistical significance was established at $p < 0.05$. Computations were performed in GraphPad Prism version 10.2.3 (GraphPad Software, San Diego, CA, USA).

RESULTS

No mortalities occurred during the acute toxicity testing across all evaluated dosage levels. Because no lethal outcomes were observed, a precise numerical LD50 could not be

derived by the AOT425StatPgm software, indicating that the oral median lethal dose of pure potassium sorbate exceeds 1,100 mg/kg in rodents under the tested conditions. Furthermore, no significant differences in body weight gain or clinical signs were noted in animals subjected to repeated doses of the pure substance compared to controls ($p > 0.05$).

In the genotoxicity assessments, the commercial formulations Golden Blue, Aquafiv, and Aquativagro exhibited no clastogenic or mutagenic potential. In the in vitro alkaline comet assay with L929 fibroblasts, neither the standard assay nor the Proteinase K-modified protocol revealed significant increases in DNA damage indices across the 1:1000, 1:500, and 1:100 dilutions compared to baseline levels. In contrast, the positive control (MMS) produced substantial DNA damage ($p < 0.0001$), validating assay responsiveness (Figure 1A–B). Similarly, in vivo evaluation via the peripheral blood micronucleus assay showed no significant increase in micronucleated polychromatic erythrocytes for any of the tested formulations (Figure 1C).

Figure 1 - Genotoxicity assessment of commercial potassium sorbate-based formulations at 1:1000, 1:500, and 1:100 dilutions

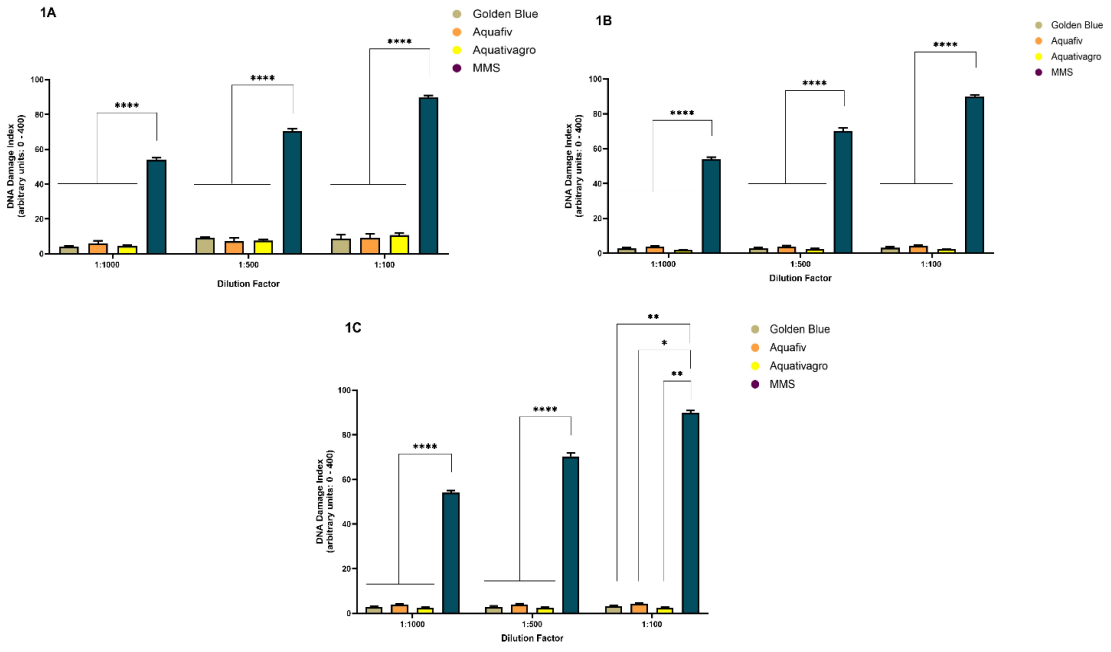

Figure 1. (A) Conventional alkaline comet assay; (B) comet assay with Proteinase K; and (C) micronucleus assay. In the comet assays, the results correspond to the DNA damage index, whereas in the micronucleus assay, they correspond to micronucleus frequency. Data are presented as mean ± standard deviation (SD). Statistical comparisons were performed using one-way ANOVA followed by Dunnett's test (****$p < 0.0001$ *vs.* MMS – methyl methanesulfonate, positive control) for parametric data, and the Kruskal–Wallis test followed by Dunn's post-hoc test (*$p < 0.05$ and **$p < 0.01$ *vs.* MMS – methyl methanesulfonate, positive control) for non-parametric data.

Hematological profiling revealed significant alterations in the erythroid and myeloid series in animals exposed to the 1:100 dilution of the commercial products. A statistically significant reduction in red blood cell count (RBC) was observed in all treatment groups

relative to the control ($p < 0.01$ and $p < 0.001$), while circulating hemoglobin concentrations (HGB) remained unaltered across all groups (Figure 2A–B). Analysis of the white blood cell series demonstrated marked leukopenia, driven by significant decreases in total leukocyte and monocyte counts ($p < 0.05$, $p < 0.01$, and $p < 0.001$), whereas lymphocyte counts remained comparable to control values (Figure 2C–E).

Figure 2 - Assessment of hematological parameters in animals exposed to commercial potassium sorbate-based formulations

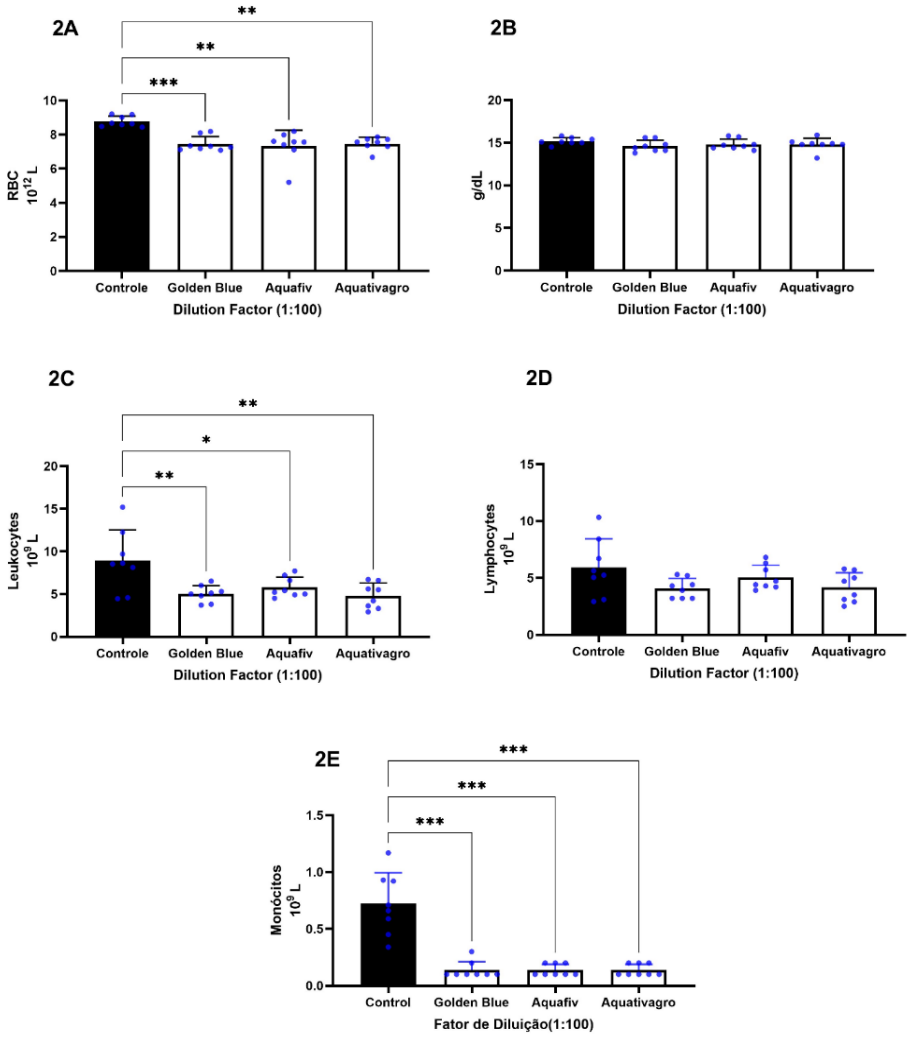

Figure 2. Assessment of hematological parameters at the 1:100 dilution. (A) Red blood cells (RBC); (B) hemoglobin (HGB); (C) total leukocytes; (D) lymphocytes; and (E) monocytes. Data are presented as mean ± standard deviation (SD). For RBC, HGB, and monocytes, the Kruskal–Wallis test followed by Dunn's post-hoc test was used (**p < 0.01 or ***p < 0.001 vs. Control group). For leukocytes and lymphocytes, one-way ANOVA followed by Dunnett's test was used (*p < 0.05 or **p < 0.01 vs. Control).

Serum biochemical analyses demonstrated distinct changes in hepatic and renal parameters. Animals exposed to the commercial formulations exhibited significant reductions in serum ALT and AST activities compared to controls ($p < 0.05$, $p < 0.001$, and $p < 0.0001$), while

total and direct bilirubin concentrations showed no significant deviations (Figure 3A–D). Regarding renal function, a statistically significant increase in serum creatinine was observed across all formulation-treated groups ($p < 0.0001$), whereas serum urea levels and fasting blood glucose concentrations remained unchanged ($p > 0.05$) (Figure 3E–F).

Figure 3 - Assessment of hepatic and renal biochemical parameters in animals exposed to commercial potassium sorbate-based formulations

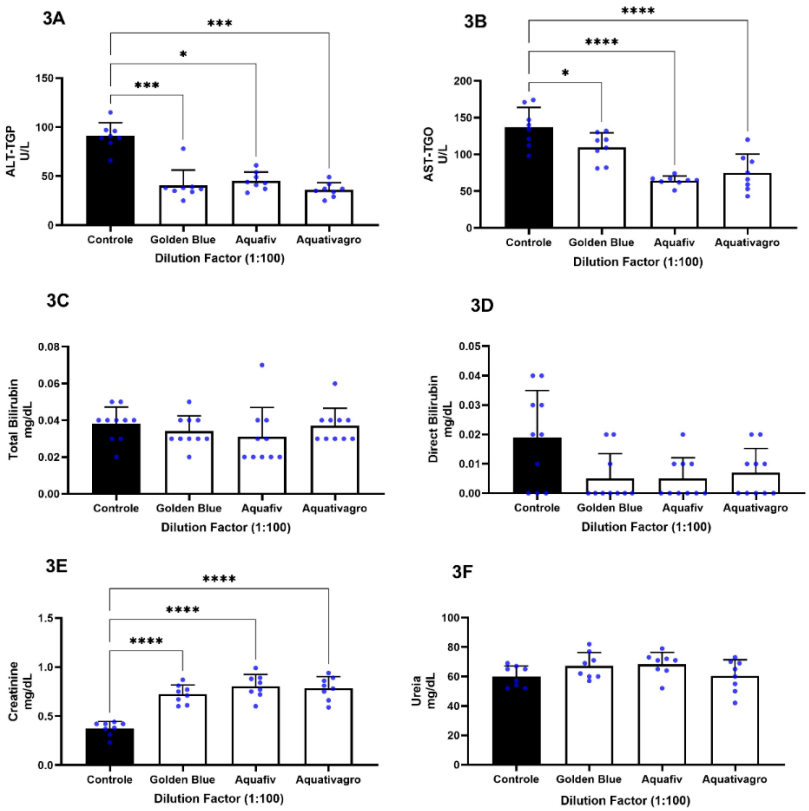

Figura 3. Assessment of biochemical parameters at the 1:100 dilution. (A) Alanine aminotransferase (ALT); (B) aspartate aminotransferase (AST); (C) total bilirubin; (D) direct bilirubin; (E) creatinine; and (F) urea. Data are presented as mean ± standard deviation (SD). For ALT, total bilirubin, and direct bilirubin, the Kruskal–Wallis test followed by Dunn's post-hoc test was used ($p < 0.05$ or $^{**}p < 0.001$ vs. Control). For AST, creatinine, and urea, one-way ANOVA followed by Dunnett's test was used ($p < 0.05$ or $^{***}p < 0.0001$ vs. Control).

Histopathological evaluation confirmed notable tissue alterations in the kidneys and livers of animals treated with the 1:100 dilution of Golden Blue (G1), Aquafiv (G2), and Aquativagro (G3) (Table 1). In the kidneys, moderate-to-severe glomerular congestion, diffuse cortical tubular epithelial swelling, vacuolar degeneration, and intraluminal hyaline casts were consistently observed across all treated groups. Notably, tubular necrosis and epithelial desquamation occurred exclusively in the Aquafiv-treated cohort (G2). In the liver, treated animals exhibited portal/centrilobular congestion, hepatocellular swelling, sinusoidal hemorrhage, Kupffer cell hyperplasia, discrete inflammatory cell infiltration, and microvesicular steatosis. The severity of microvesicular steatosis progressed from mild in G1

(Golden Blue) to moderate in G2 (Aquafiv) and marked/severe in G3 (Aquativagro), without evidence of confluent hepatocellular necrosis.

Tabela 1. Semiquantitative histopathological assessment of the liver and kidneys of animals exposed to commercial potassium sorbate-based formulations

Lesion	Golden Blue (G1)	Aquafiv (G2)	Aquativagro (G3)
KIDNEYS			
Glomerular congestion	+++	++	++
Cortical tubular epithelial swelling	++	++	++
Tubular vacuolar degeneration	++	++	+++
Tubular epithelial necrosis or desquamation	-	++	-
Hyaline casts	+	++	++
Interstitial inflammatory infiltrate	-	-	-
LIVER			
Portal/centrilobular congestion	+	+	+
Hepatocellular swelling	++	++	++
Hydropic degeneration	-	-	-
Kupffer cell hyperplasia	+	+	+
Sinusoidal hemorrhage	++	++	++
Inflammatory foci/cells	+	++	++
Microvesicular steatosis	+	++	+++
Focal hepatocellular necrosis	-	-	-

Table 1. Animals were exposed to the commercial formulations at the 1:100 dilution. Symbols: – (absent/not observed); + (mild); ++ (moderate); +++ (severe).

DISCUSSION

The absence of mortality following acute oral administration confirms previous evaluations establishing pure potassium sorbate as a chemical agent with high biological tolerance and low acute toxicity (American College of Toxicology, 1988). The determination of an oral LD50 exceeding 1,100 mg/kg in mice aligns with classical safety thresholds documented for sorbic acid salts in mammalian models.

The lack of detectable clastogenic or aneugenic damage in both in vitro (comet assay) and in vivo (micronucleus test) models exposed to Golden Blue, Aquafiv, and Aquativagro indicates that these commercial formulations do not directly induce primary DNA lesions or chromosomal fragmentation under the tested dilution regimens (Kirkland et al., 2007; Hayashi, 2016). However, the absence of direct genotoxicity does not preclude systemic and tissue-level

toxicological responses, as evidenced by the hematological, biochemical, and histopathological findings.

The observed decrease in total erythrocyte count (RBC) without a corresponding decline in hemoglobin concentration indicates an early impact on circulating red cell mass or bone marrow erythropoiesis. Although isolated RBC reductions without concurrent hemoglobin alterations do not fulfill the complete diagnostic criteria for anemia, they highlight subtle hematological stress that warrants careful clinical consideration alongside full erythrocyte indices (Thrall et al., 2012). Similarly, significant reductions in total circulating leukocytes and monocytes point to mild immunosuppression or altered leukocyte homing. These responses are consistent with reports indicating that industrial additive mixtures can modulate immune cell kinetics and inflammatory homeostasis (Abd-Elhakim et al., 2023).

In the biochemical profile, the reduction in serum ALT and AST activity deviates from classic hepatocellular cytolysis patterns, which are typically characterized by enzyme leakage and marked transaminase elevations (Abd-Elhakim et al., 2023). Decreased circulating transaminase levels may stem from impaired basal hepatic enzyme synthesis or analytical interference caused by formulation excipients, underscoring the critical necessity of integrating biochemical markers with histopathological evaluations (Kaneko et al., 2008).

The significant rise in serum creatinine, occurring alongside stable urea levels, correlates directly with the structural changes detected in renal tissue, including glomerular congestion, tubular swelling, vacuolar degeneration, and intraluminal hyaline casts. These concurrent findings provide functional and morphological evidence of tubular injury and altered renal clearance under formulation exposure (Latimer, 2011; Wu et al., 2025). Furthermore, the observation of microvesicular steatosis and hepatocellular swelling in liver tissue reinforces the concept that formulated products containing industrial surfactants and stabilizing agents exhibit toxicodynamic behaviors distinct from those of the pure preservative salt alone (Zhang et al., 2025).

CONCLUSION

Pure potassium sorbate demonstrated low acute and subchronic oral toxicity in rodents, causing no mortality or significant changes in body weight gain. Although the commercial formulations Golden Blue, Aquafiv, and Aquativagro showed no genotoxic effects in comet and micronucleus assays, their subchronic administration induced notable hematological shifts, serum creatinine elevations, and distinct histopathological lesions in both renal and hepatic

parenchymas, primarily characterized by tubular degeneration and microvesicular steatosis. These findings demonstrate that carrier matrices and adjuvants present in commercial formulations can modify the biological behavior of preservatives, underscoring the necessity for comprehensive toxicological monitoring of commercial preparations.

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