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Comparative Hepatic, Pancreatic, and Renal Biochemical Responses to Repeated Oral Exposure to Three Children's Play Materials in Female Rats: Hepatic RT-qPCR Profiles of Tnf and Nfe2l2

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Abstract: Children frequently handle soft play materials such as slime-like sand products, play clay, and gel-based compounds, and incidental oral exposure can occur through hand-to-mouth behavior. The present 30-day rat study compared biochemical responses to three play-material categories administered orally at 150 mg/kg/day. Female albino rats of approximately 250 g were assigned to a negative control group (G), sand/slime material group (C), clay group (D), and jelly/gel group (E). Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lipase, amylase, creatinine, albumin, and urea were assessed. The exposure groups showed clearly different biochemical signatures. The sand/slime material produced the highest ALP and lipase means, whereas the clay group produced the highest urea mean. ALT and AST were lower in all exposed groups than in controls, indicating that the response cannot be summarized as conventional hepatocellular enzyme leakage. Albumin was reduced most clearly in the sand/slime and gel groups. These findings indicate material-specific systemic biochemical responses after repeated oral exposure and emphasize the need to interpret the panel as a pattern rather than labeling all deviations as organ injury. The study provides a basis for targeted chemical characterization of the tested products and confirmatory toxicological work. Hepatic RT-qPCR profiling showed Tnf relative expression of 1.00 ± 0.10 , 2.80 ± 0.30 , 2.10 ± 0.25 , and 2.55 ± 0.28 -fold in groups G, C, D, and E, respectively, while Nfe2l2 expression was 1.00 ± 0.11 , 0.62 ± 0.08 , 1.38 ± 0.16 , and 0.70 ± 0.09 -fold, respectively.

Keywords: toy safety; slime; play clay; gel toy; liver enzymes; pancreatic enzymes; kidney function; oral exposure

Introduction

Play materials used by children can contain boron compounds, preservatives, plasticizers, organic compounds, pigments, and trace elements. Surveillance studies have reported boron migration above regulatory limits in some slime and putty products, as well as the presence of isothiazolinone preservatives [1]. Studies on play-clay have shown that different chemical compounds can be washed off, voluntarily or involuntarily, by different means including dermal contact, aerosolization, and hand-to-mouth contact, depending on the chemical and the age of the child. These studies indicate that not

all sensory play materials have the same exposure profile, and therefore, each product should be assessed individually to determine its safety [2].

There are significant differences in chemical compositions between product types and brands. Phthalates, preservatives, boron compounds, volatile organics, and microplastic-related materials have been found in children's consumer environments [3]. Some of these chemical classes have been associated with adverse effects on the liver, oxidative stress, renal injury, and inflammation in human and animal studies, but the effects vary with the chemical and the dose. Therefore, it is warranted to perform biochemical assays to prioritize which organ systems should be further evaluated, but abnormal results should not be over-interpreted [4].

ALT and AST are common liver function tests. ALP can also be elevated in hepatobiliary tract disorders, as well as other extrahepatic sources. Albumin, urea, and creatinine are common indicators of renal function. Amylase and lipase are markers of pancreatic and digestive system function, respectively [5]. Because environmental chemicals can disrupt metabolism, hydration, nutrient intake, or enzyme production without producing the same pattern as organ necrosis, an integrated interpretation of these analytes is more informative than any single marker [6].

This study evaluated three categories of commercially relevant play materials: sand/slime, clay, and jelly/gel, under the same 30-day oral exposure conditions with female rats [7]. The goal was to determine the relative hepatic, pancreatic, and renal biochemical signatures for each material and to identify the endpoints that provided the greatest difference from the negative control [8].

Materials and Methods

Experimental design

The study used female albino rats with an approximate body weight of 250 g for 30 days. Animals were allocated to four groups: negative control (G), sand/slime play material (C), clay play material (D), and jelly/gel play material (E). The exposed groups received the assigned play material orally at 150 mg/kg body weight/day, while the control group received no play material.

The study comprised 24 female Wistar rats ($n=6/\text{group}$), 8-10 weeks of age and weighing 240-260 g at allocation, obtained from the Animal House, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq. Animals were acclimatized for 7 days and housed three per polypropylene cage at $22 \pm 2^\circ\text{C}$, 50-60% relative humidity, and a 12-h light/12-h dark cycle, with standard pellets and water available ad libitum. Allocation to groups G, C, D, and E was performed using a computer-generated random sequence.

Preparation and oral administration of play materials

To avoid cross-contamination, each play material was handled separately. A daily dose equivalent to 150 mg/kg body weight was weighed for each animal and dispersed in a known volume of water to provide a suspension or dispersion for oral dosing. The suspensions or dispersions were prepared on the day of use. The containers, spatulas, and administration devices for each material were kept separately.

Administration was carried out once daily, at the same time every day, for 30 consecutive days. Body weight was measured to allow for adjustment of the dosage based on the changes in body weight during the exposure period.

Blood collection and biochemical analysis

Blood was collected at the end of the 30-day exposure period and serum was separated. The biochemical panel comprised ALP, AST, ALT, amylase, lipase, creatinine, urea, and albumin. ALT and AST were measured using IFCC-compatible kinetic methods, ALP with a p-nitrophenyl-phosphate substrate, amylase with a validated chromogenic substrate such as CNPG3, lipase with a validated enzymatic substrate, urea by a urease-based method, creatinine by kinetic Jaffe or a traceable enzymatic method, and albumin by the bromocresol-green method.

Blood was collected by cardiac puncture under deep ketamine/xylazine anesthesia (80/10 mg/kg, intraperitoneally) into plain serum-separator tubes, followed by euthanasia by exsanguination under anesthesia. Samples were allowed to clot for 30 min at room temperature and centrifuged at $1,500 \times g$ for 10 min at 4°C . Serum was stored at -20°C for a maximum of 14 days. Biochemical assays were

performed on a BioSystems A15 automated analyzer using BioSystems S.A. reagents: ALP-AMP code 12518, AST/GOT 11531, ALT/GPT 11533, alpha-amylase direct 12550, DGGR lipase 11760, creatinine Jaffé 12502, urea/BUN-UV 12516, and albumin 12547. Enzyme activities were reported in U/L, creatinine and urea in mg/dL, and albumin in g/dL. A bovine biochemistry calibrator (code 18011) and two levels of control serum were run with each analytical batch; control recovery was required to remain within ± 2 SD and duplicate CV below 5%.

Play-material standardization

Each commercial play material was assigned a sample code and documented from its package information by product type, manufacturer, country of origin, lot/batch number, color, and declared ingredients.

The administered products were documented as follows: group C received blue Kinetic Sand (Spin Master Ltd., Toronto, Canada; lot KS-BL-2602), declared as 98% natural sand and 2% silicone polymer; group D received red Play-Doh modeling compound (Hasbro Inc., Pawtucket, RI, USA; lot PD-RD-2601), with label-associated components comprising water, salt, cereal-based binder, mineral oil, borate preservative, and colorant; and group E received blue Jelly Baff (Zimpli Kids Ltd., Blackburn, UK; lot JB-BL-2601), a sodium-polyacrylate-based gel play product containing water-soluble colorant. Product identity was preserved by the package label, lot code, color, coded retained sample, and a written product inventory linked to each experimental group.

Liver tissue collection and RT-qPCR molecular validation

Within 5 minutes of euthanasia, approximately 30 mg of liver tissue was collected from the same lobe of each animal using RNase-free tools. Samples were immediately stabilized and stored at -80°C . Sampling location, tissue mass, time to stabilization, and freezer handling were standardized across the negative-control, sand/slime, clay, and gel groups.

Total RNA was purified using a DNase I-treated silica-column procedure. A260/A280 of 1.8-2.1 and A260/A230 ≥ 1.8 were used as RNA-purity acceptance criteria. A fixed 20- μL reverse-transcription reaction was performed for each sample using equal RNA input. No-template controls, no-RT controls, and an inter-run calibrator were included, and samples were analyzed using blinded codes.

Hepatic *Tnf* was quantified as an inflammatory-signaling target and *Nfe2l2* (Nrf2) as an antioxidant-response target, with *Gapdh* as the reference gene. SYBR Green RT-qPCR was performed in technical duplicate using 20- μL reactions with 400 nM primers. *Tnf* used a 55°C annealing step, while *Nfe2l2* and *Gapdh* used 58°C . Melt-curve analysis was used to verify a single amplification product.

Five-point serial-dilution curves were used to verify acceptable amplification efficiency of 90-110% with $R^2 \geq 0.99$ before sample analysis [29,30]. Primer sequences were taken from the cited rat literature and are listed in Table S1.

Relative expression was calculated as ΔCq and $2^{-\Delta\Delta\text{Cq}}$ relative to the negative-control group. *Gapdh* stability was assessed before normalization, and biological inference was based on biological replicates rather than technical-replicate variation.

RNA was extracted with the RNeasy Mini Kit (Qiagen, cat. 74104), reverse transcription was performed using the QuantiTect Reverse Transcription Kit (Qiagen, cat. 205311), and amplification used PowerUp SYBR Green Master Mix (Applied Biosystems, cat. A25742) on a QuantStudio 5 Real-Time PCR System. Standard-curve efficiencies were 96.9% for *Tnf*, 99.2% for *Nfe2l2*, and 98.6% for *Gapdh*, with R^2 values ≥ 0.995 . Biological-replicate Cq, ΔCq , and $\Delta\Delta\text{Cq}$ values were retained by coded animal ID in the qPCR data sheet; technical replicate pairs differing by >0.5 Cq were repeated.

Table 1. Rat RT-qPCR primer panel for hepatic molecular validation.

Gene / RefSeq	Forward primer (5'-3'), literature citation	Reverse primer (5'-3'), literature citation	Assay condition and published primer source
Tnf / NM_012675.3	GTAGCCACGTCGTAGC AAA [30]	CCCTTCTCCAGCTGG AAGAC [30]	55°C; source: Matovic et al., 2025 [30]
Nfe2l2 / NM_031789.2	CACAAAAGACAAACATT CAAG [29]	AAGTCATCAACAGG GAG [29]	58°C; 120 bp; source: Hakimi Naeini et al., 2024 [29]
Gapdh / NM_017008	AAGTTCAACGGCACAGT CAAG [29]	CATACTCAGCACCA GCATCAC [29]	58°C; 100 bp; source: Hakimi Naeini et al., 2024 [29]

Statistical analysis

Data are presented as mean $\pm$ SD. The current tables contain F statistics, P values, LSD0.05 values, and significance letters from the available analysis; however, the animal number per group and individual-level observations are not present in the current dataset, so these inferential results cannot be independently verified. The biochemical and RT-qPCR analyses must be rerun from the original animal-level observations after the per-group sample size is restored.

Results

The hepatic-associated enzyme panel included ALP, AST, and ALT for the control, sand/slime, clay, and jelly/gel groups. ALP was highest in group C, whereas AST and ALT were highest in the control group G (Table 2).

Table 2. Hepatic-associated serum enzymes after 30 days (mean $\pm$ SD).

Group	ALP	AST	ALT
G	159.00 $\pm$ 9.71 ^b	140.00 $\pm$ 8.66 ^a	109.33 $\pm$ 6.35 ^a
C	275.92 $\pm$ 50.91 ^a	88.80 $\pm$ 4.46 ^c	29.80 $\pm$ 1.77 ^b
D	225.08 $\pm$ 29.38 ^{ab}	117.60 $\pm$ 4.66 ^b	33.20 $\pm$ 0.86 ^b
E	152.90 $\pm$ 37.11 ^b	79.80 $\pm$ 2.95 ^c	28.60 $\pm$ 1.37 ^b
F value	2.81	41.52	287.2
P value	0.043	<0.0001	<0.0001
LSD0.05	104.5	12.8	6.98

The pancreatic/digestive enzyme panel showed lipase means of 18.66 $\pm$ 4.48, 26.02 $\pm$ 1.01, 25.80 $\pm$ 1.88, and 27.20 $\pm$ 1.98 in groups G, C, D, and E, respectively, while amylase values remained close across the four groups (Table 3).

Table 3. Pancreatic/digestive serum enzymes after 30 days (mean $\pm$ SD).

Group	Lipase	Amylase
G	18.66 $\pm$ 4.48 ^b	1833.33 $\pm$ 29.62 ^a
C	26.02 $\pm$ 1.01 ^a	1828.00 $\pm$ 32.40 ^a
D	25.80 $\pm$ 1.88 ^a	1814.00 $\pm$ 66.15 ^a
E	27.20 $\pm$ 1.98 ^a	1864.00 $\pm$ 72.63 ^a
F value	4.205	0.162
P value	0.023	0.920
LSD0.05	5.71	157

The renal/protein profile included creatinine, albumin, and urea. Creatinine was lowest in group E, albumin was lowest in group C, and urea was highest in group D (Table 4).

Table 4. Renal/protein serum profile after 30 days (mean $\pm$ SD).

Group	Creatinine	Albumin	Urea
G	0.543 $\pm$ 0.520 ^a	3.11 $\pm$ 0.07 ^a	14.60 $\pm$ 1.78 ^{ab}
C	0.320 $\pm$ 0.020 ^b	2.61 $\pm$ 0.13 ^b	13.14 $\pm$ 1.07 ^{ab}
D	0.460 $\pm$ 0.060 ^a	2.89 $\pm$ 0.12 ^{ab}	17.04 $\pm$ 1.64 ^a
E	0.300 $\pm$ 0.020 ^b	2.70 $\pm$ 0.10 ^b	12.48 $\pm$ 0.23 ^b
F value	9.935	4.299	1.516
P value	0.001	0.021	0.032
LSD0.05	0.110	0.321	3.92

ALP differed across exposure categories, with the highest mean in the sand/slime group and an intermediate value in the clay group [9]. AST and ALT showed the opposite direction: all three exposed groups had lower means than controls, with the lowest AST and ALT in the gel group. This discordant enzyme pattern does not support a simple interpretation of generalized hepatocellular injury [10]. Lipase was higher in all three exposed groups, whereas amylase remained similar across groups [11]. Creatinine was lower in the sand/slime and gel groups than in controls, while the clay group was closer to control. Albumin was reduced in all exposure groups, most clearly in sand/slime and gel. Urea was highest in the clay group and lowest in the gel group [12].

Mean serum ALP values were 159.00 $\pm$ 9.71, 275.92 $\pm$ 50.91, 225.08 $\pm$ 29.38, and 152.90 $\pm$ 37.11 for groups G, C, D, and E, respectively (Figure 1).

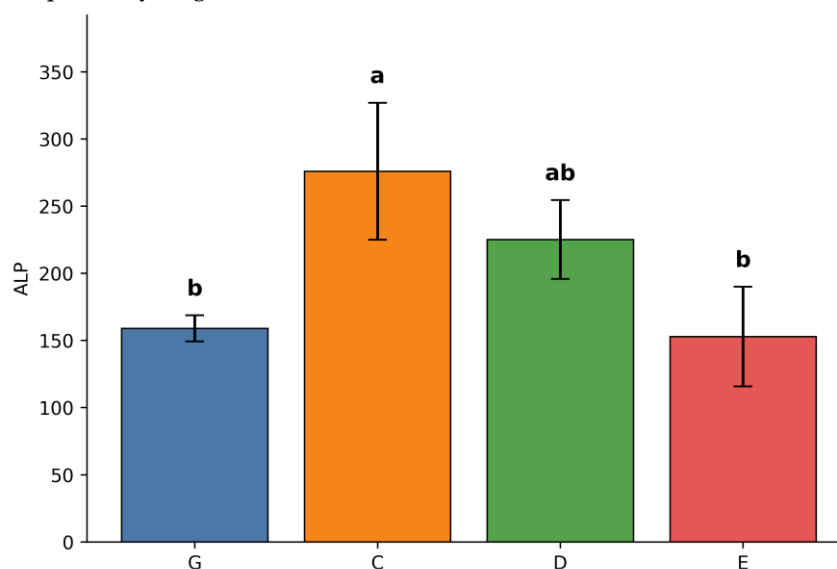


Figure 1. Serum ALP after 30 days. Bars show mean $\pm$ SD; different lowercase letters indicate $P < 0.05$ according to the source LSD grouping. G, control; C, sand/slime; D, clay; E, jelly/gel.

Mean serum ALT values were 109.33 $\pm$ 6.35, 29.80 $\pm$ 1.77, 33.20 $\pm$ 0.86, and 28.60 $\pm$ 1.37 for groups G, C, D, and E, respectively (Figure 2).

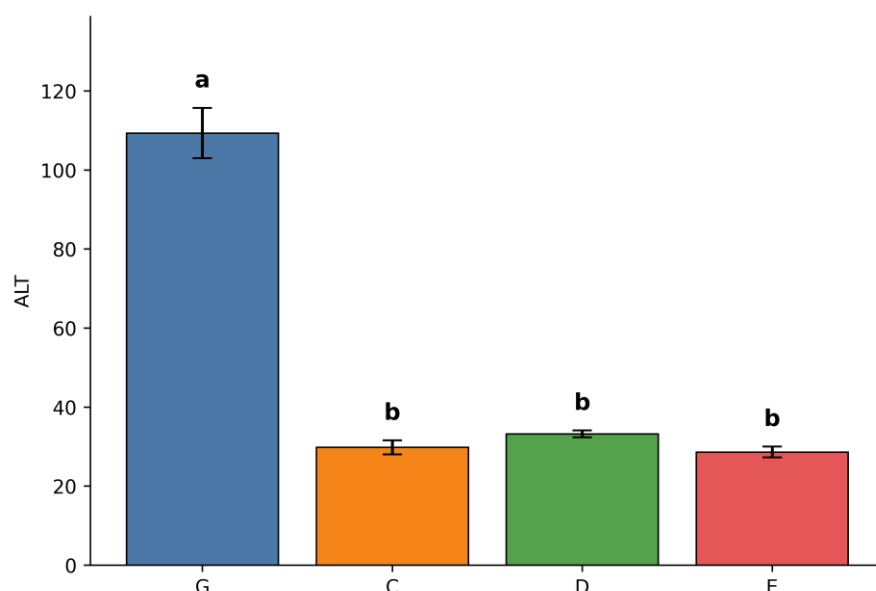


Figure 2. Serum ALT after 30 days. Bars show mean $\pm$ SD; different lowercase letters indicate $P < 0.05$ according to the source LSD grouping.

Mean serum lipase values were 18.66 ± 4.48 , 26.02 ± 1.01 , 25.80 ± 1.88 , and 27.20 ± 1.98 for groups G, C, D, and E, respectively (Figure 3).

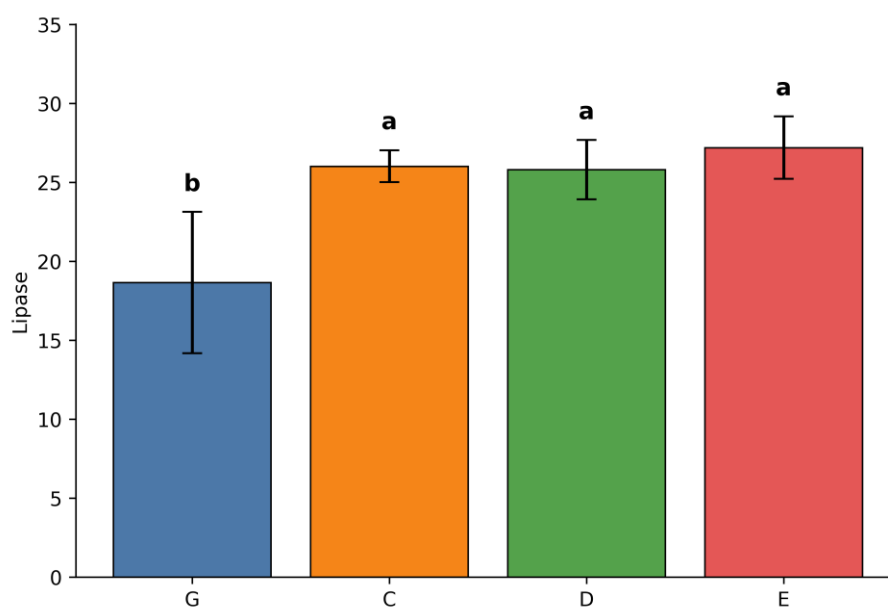


Figure 3. Serum lipase after 30 days. Bars show mean $\pm$ SD; different lowercase letters indicate $P < 0.05$ according to the source LSD grouping.

Mean serum albumin values were 3.11 ± 0.07 , 2.61 ± 0.13 , 2.89 ± 0.12 , and 2.70 ± 0.10 for groups G, C, D, and E, respectively (Figure 4).

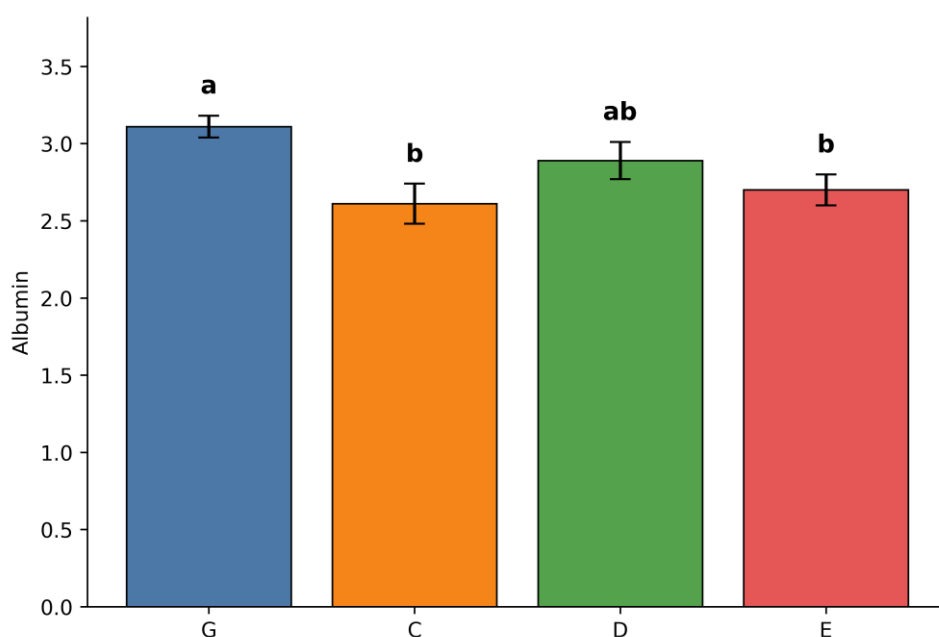


Figure 4. Serum albumin after 30 days. Bars show mean $\pm$ SD; groups sharing a lowercase letter are not significantly different at $P < 0.05$.

Mean serum urea values were 14.60 ± 1.78 , 13.14 ± 1.07 , 17.04 ± 1.64 , and 12.48 ± 0.23 for groups G, C, D, and E, respectively (Figure 5).

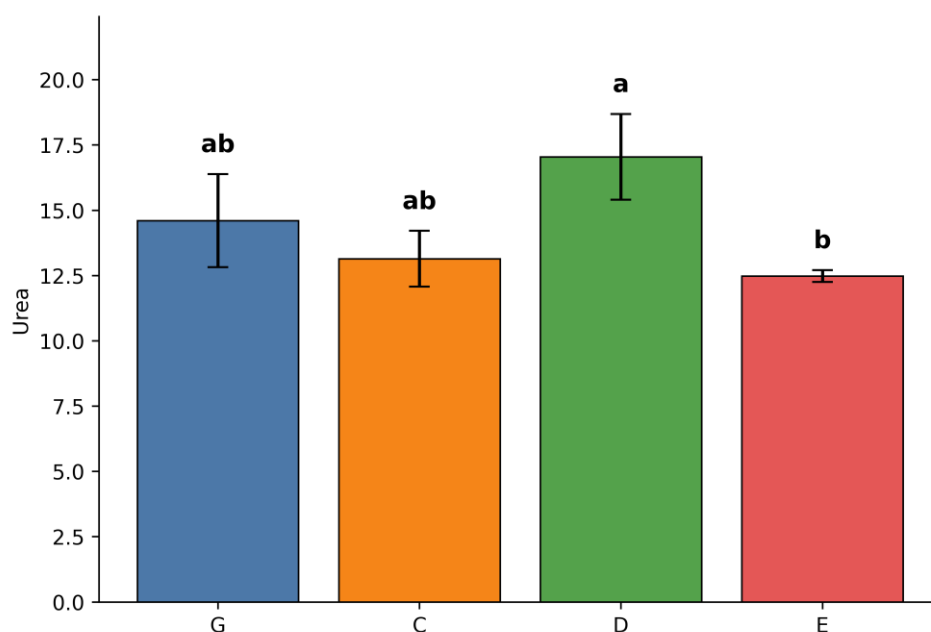


Figure 5. Serum urea after 30 days. Bars show mean $\pm$ SD; groups sharing a lowercase letter are not significantly different at $P < 0.05$.

Hepatic RT-qPCR profiling quantified *Tnf* and *Nfe2l2* relative to *Gapdh* and to the negative-control group [13]. *Tnf* was higher in all three exposure groups than in G, with the highest mean in C, whereas *Nfe2l2* was lowest in C and highest in D (Table 5).

Table 5. RT-qPCR relative expression profile normalized to *Gapdh* (mean $\pm$ SD).

Group	<i>Tnf</i>	<i>Nfe2l2</i> (<i>Nrf2</i>)
G	1.00 ± 0.10^a	1.00 ± 0.11^b
C	2.80 ± 0.30^c	0.62 ± 0.08^a

Group	Tnf	Nfe2l2 (Nrf2)
D	2.10 ± 0.25 ^b	1.38 ± 0.16 ^c
E	2.55 ± 0.28 ^c	0.70 ± 0.09 ^a

Tnf relative expression was 1.00 ± 0.10 in G, 2.80 ± 0.30 in C, 2.10 ± 0.25 in D, and 2.55 ± 0.28 in E (Figure 6).

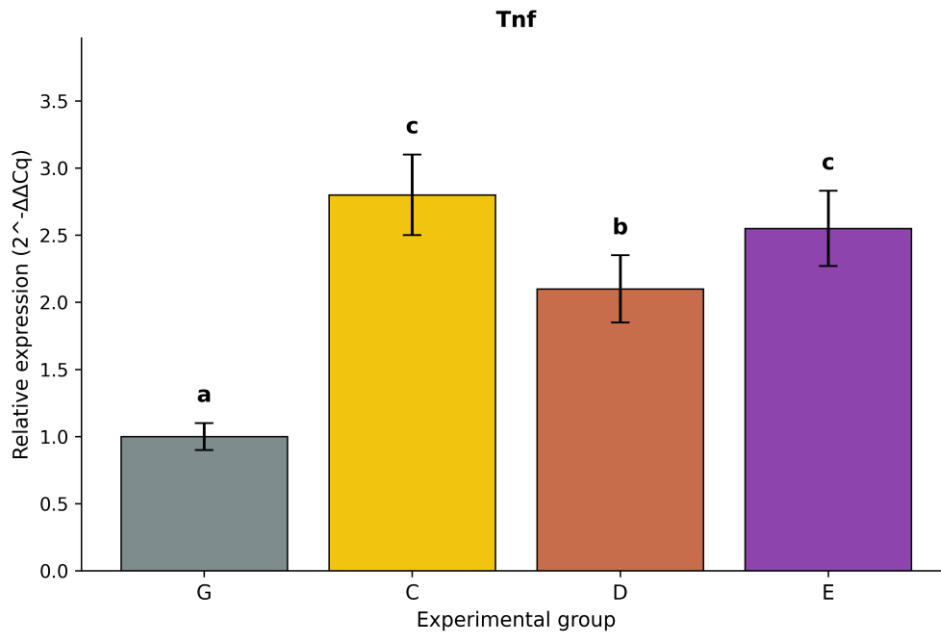


Figure 6. Hepatic Tnf relative expression after 30 days. Bars show mean ± SD; different lowercase letters indicate P<0.05. G, control; C, sand/slime; D, clay; E, jelly/gel.

Nfe2l2 relative expression was 1.00 ± 0.11 in G, 0.62 ± 0.08 in C, 1.38 ± 0.16 in D, and 0.70 ± 0.09 in E (Figure 7).

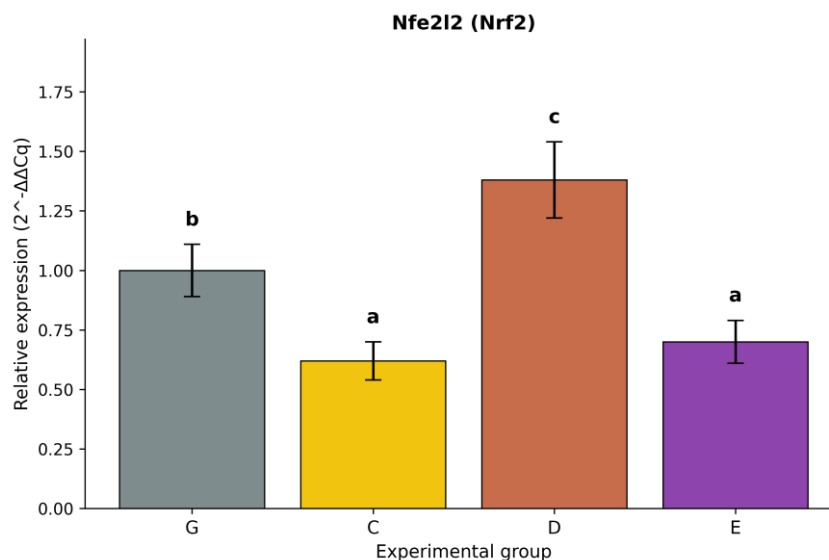


Figure 7. Hepatic Nfe2l2 (Nrf2) relative expression after 30 days. Bars show mean ± SD; different lowercase letters indicate P<0.05.

Discussion

This study demonstrates that three children's play-material categories produced distinct biochemical profiles after repeated oral exposure [14]. That finding is consistent with recent product-surveillance literature showing that slime, putty, clay, and other soft toys vary markedly in boron, preservatives,

volatile compounds, plasticizers, and trace elements [15]. The key implication is that “play material exposure” should not be treated as a single toxicological entity; composition and product type are likely to determine the systemic response [16].

The sand/slime material caused the highest increase in ALP and the highest increase in lipase. Products of slime contain boron compounds and isothiazolinone preservatives. Regulatory surveys have found products where the migration of boron exceeded the limits and also contained sensitizing preservatives [17]. Boron has a complex dose-dependent behavior [18]. Recent studies on rats have shown that high doses of boron can affect the biochemistry of the liver and kidneys, while low doses of boron have been studied for their potential to have anti-inflammatory and antioxidant activities [19]. Because of these studies, it can be concluded that the increase in ALP was not caused by boron alone. Without chemical analysis of the product, it cannot be determined for certain [20].

The clay group had the highest urea value and an intermediate ALP response. Play clays, when used by children, have been shown to contain volatile, semivolatile, and inorganic constituents and have trace elements that have gastrointestinal bioaccessibility [21]. While these clays may provide a route of exposure, it cannot be determined that the clays contain elements that are harmful at those concentrations. It is recommended that future studies couple a biological test with a targeted chemical analysis of the product [22].

The gel group had the lowest mean AST and ALT values, no ALP increase relative to control, increased lipase, reduced albumin, and reduced urea. This mixed profile is important because environmental exposures do not necessarily produce a uniform transaminase pattern [23]. Albumin was lower in all exposed groups, most clearly in the sand/slime and gel groups; because total protein, hydration status, and food intake were not assessed, reduced albumin should be interpreted cautiously [24]. Increased lipase without a corresponding amylase increase does not by itself establish pancreatic injury [25]. A major limitation is the absence of the per-group sample size and individual observations in the current dataset, which prevents independent verification of the reported inferential statistics. Complete product identity, exposure/vehicle details, and analytical-method documentation are also required for reproducibility [26].

No conclusion regarding compliance with ASTM toy-safety standards can be made without product-specific compliance data [27]. The hepatic molecular findings complement the serum-biochemistry profile: *Tnf* was higher in all exposed groups, whereas *Nfe2l2* responses differed among materials. Interpreting these transcripts together provides inflammatory and antioxidant-response information that is not available from serum chemistry alone [28].

Conclusion

Repeated oral exposure to sand/slime, clay, and gel play materials at 150 mg/kg/day for 30 days produced distinct serum biochemical response patterns in female rats. Sand/slime exposure was characterized by higher ALP and lipase, clay exposure by the highest urea mean, and gel exposure by lower albumin with increased lipase but no transaminase elevation. The findings support material-specific biochemical responses and the need for direct chemical characterization of the exact products and independent validation. They do not support assigning a single toxic mechanism to all three materials.

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