Appendix A: Literature search for specific toxicology studies with novel supplement formulations

In this guide

Searches for studies investigating the toxicity of vitamin C, CBD, and curcumin in novel formulations were conducted in PubMed using the search strings listed in table 1.

Table 1. Search strings and number of total and relevant results.

Search string	Results	Relevant
“Vitamin C” AND “toxicity” AND “encapsulated”	7	0
“Vitamin C” AND “toxicity” AND “liposomal”	5	0
“Vitamin C” AND “toxicity” AND “micelles”	6	0
“Vitamin C” AND “toxicity” AND “emulsion”	3	0
“CBD” AND “toxicity” AND “encapsulated”	1	0
“CBD” AND “toxicity” AND “liposomal”	0	0
“CBD” AND “toxicity” AND “micelles”	0	0
“CBD” AND “toxicity” AND “emulsion”	0	0
“Curcumin” AND “toxicity” AND “encapsulated”	121	7
“Curcumin” AND “toxicity” AND “liposomal”	33	5
“Curcumin” AND “toxicity” AND “micelles”	84	8
“Curcumin” AND “toxicity” AND “emulsion”	27	3

PM = PubMed; WoS = Web of Science.

Relevant results were only retrieved for novel formulations of curcumin. A total of 23 studies were identified, 11 of which were *in vitro* studies and 11 of which were *in vivo* studies. Three studies were performed in human subjects and are reviewed in the main paper. A few of the relevant hits were retrieved by more

than one search string and in these cases such results were omitted from the ‘relevant’ count in the subsequent strings such that all ‘relevant’ counts are unique. Publications investigating the toxicology of novel curcumin formulations *in vitro* and *in vivo* are briefly summarised table 2 and the full references are listed below table 2.

Table 2. Summary of studies investigating the toxicity of novel curcumin formulations.

Formulation - In Vitro	**System**	**Key findings**	**Study**
Curcumin nano-blisomes	Non-cancer cell line (Wi-38l)	Lower cytotoxicity vs unformulated curcumin	Abbas *et al*. 2022
Micellar curcumin	Immortalised fibroblasts Glioblastoma LN229 Human endothelial cell line Primary vascular endothelial cells Primary smooth muscle cells Primary pericytes	Reduced cell viability. Reversible genotoxicity (comet assay). Similar efficacy of native vs. micellar curcumin.	Beltzig *et al*. 2021
Liposomal curcumin	Human lymphocytes EBV-transformed B-cells (LCL)	Empty DMPC liposomes toxic. Liposomal curcumin inhibited LCL proliferation.	Chen *et al*. 2009

Micellar curcumin	Breast tumor cell line Human stromal cells Zebrafish embryotoxicity assay	Induced apoptosis in tumour cells and spheroids. Reduced viability in stromal cells. Toxicity to zebrafish embryo development. Micellar curcumin more toxic.	Do *et al*. 2022
Curcumin chitosan nanoparticles	Cervical tumour cells VERO cells	Cytotoxicity to tumour cells. Biocompatible with VERO cells.	Facchi *et al*. 2019
Liposomal curcumin	Human synovial fibroblasts Mouse macrophages	Liposomal curcumin less toxic to cells.	Kloesch *et al*. 2016
Curcumin microemulsion	HepG2 cells	Cytotoxicity to HepG2 cells, greater with smaller emulsion droplet size.	Lin *et al*. 2014
Micellar curcumin	HepG2 cells	Cytotoxicity to HepG2 cells.	Phan *et al*. 2016
Solid lipid curcumin nanoparticles	3T3 fibroblasts	Reduction in cell viability and alteration of lipid profile (dependent upon particle composition).	Rosa *et al*. 2022

Liposomal curcumin	Red blood cells in vitro	Dose-dependent echinocyte formation and increases in mean cellular volume.	Storka *et al*. 2013
Liposomal solid curcumin gels	Huh7it cell line	Non-cytotoxic.	Yusuf *et al*. 2022
Formulation - In Vivo	**System**	**Key findings**	**Study**
Curcumin PLGA nanoparticles	Mice RAW 264.7 cell line	Increased lymphocytes. No changes in hepatotoxic biomarkers. Higher toxicity in RAW 264.7 cells at higher concentrations, but not at lower concentrations.	Busari *et al*. 2017
Curcumin-loaded hydrogel nanoparticles	"*in vivo*" [abstract only]	Low toxicity. No genotoxicity observed.	Dandekar *et al*. 2010
Hydrogenated curcumin	Sprague Dawley rats	No treatment related toxicity.	Gopi *et al*. 2016
Alginate-curcumin conjugate; micelle forming	Mouse tumour models.	No toxicity observed in blood parameters, histology, comet assay, or cytokine levels.	Karabasz *et al*. 2019
Nano-micelle curcumin	Male Wistar rats	Testicular toxicity observed; DNA damage.	Moshari *et al* . 2017

Nano-micelle curcumin	Male Wistar rats	Testicular toxicity observed; suppression of spermatogenesis; DNA damage.	Radmanesh *et al*. 2021
Nano-liposome curcumin with tetrandrine	Zebrafish	No developmental toxicity.	Song *et al*. 2022
Chitosan solid lipid nanoparticle curcumin with sulforaphane	BALB/c mice	No toxicity in acute, subacute, or chronic tests.	Thakkar *et al* . 2016
Micellar curcumin	Male Wistar rats	No haematopoietic or liver tissue toxicity.	Tzankova *et al*. 2016

Full references

In vitro

Abbas, H., El-Feky, Y.A., Al-Sawahli, M.M., El-Deeb, N.M., El-Nassan, H.B., Zewail, M., 2022. Development and optimization of curcumin analog nano-bilosomes using 21.31 full factorial design for anti-tumor profiles improvement in human hepatocellular carcinoma: in-vitro evaluation, in-vivo safety assay. Drug Deliv 29, 714–727. https://doi.org/10.1080/10717544.2022.2044938

Beltzig, L., Frumkina, A., Schwarzenbach, C., Kaina, B., 2021. Cytotoxic, Genotoxic and Senolytic Potential of Native and Micellar Curcumin. Nutrients 13, 2385. https://doi.org/10.3390/nu13072385

Chen, C., Johnston, T.D., Jeon, H., Gedaly, R., McHugh, P.P., Burke, T.G., Ranjan, D., 2009. An in vitro study of liposomal curcumin: stability, toxicity and biological activity in human lymphocytes and Epstein-Barr virus-transformed human B-cells. Int J Pharm 366, 133–139. https://doi.org/10.1016/j.ijpharm.2008.09.009

Do, X.-H., Hoang, M.H.T., Vu, A.-T., Nguyen, L.-T., Bui, D.T.T., Dinh, D.-T., Nguyen, X.-H., Than, U.T.T., Mai, H.T., To, T.T., Nguyen, T.N.H., Hoang, N.T.M., 2022.

Differential Cytotoxicity of Curcumin-Loaded Micelles on Human Tumor and Stromal Cells. Int J Mol Sci 23, 12362. https://doi.org/10.3390/ijms232012362

Facchi, S.P., Scariot, D.B., Bueno, P.V.A., Souza, P.R., Figueiredo, L.C., Follmann, H.D.M., Nunes, C.S., Monteiro, J.P., Bonafé, E.G., Nakamura, C.V., Muniz, E.C., Martins, A.F., 2016. Preparation and cytotoxicity of N-modified chitosan nanoparticles applied in curcumin delivery. Int J Biol Macromol 87, 237–245. https://doi.org/10.1016/j.ijbiomac.2016.02.063

Kloesch, B., Gober, L., Loebsch, S., Vcelar, B., Helson, L., Steiner, G., 2016. In Vitro Study of a Liposomal Curcumin Formulation (Lipocurc™): Toxicity and Biological Activity in Synovial Fibroblasts and Macrophages. In Vivo 30, 413–419.

Lin, C.-C., Lin, H.-Y., Chi, M.-H., Shen, C.-M., Chen, H.-W., Yang, W.-J., Lee, M.-H., 2014. Preparation of curcumin microemulsions with food-grade soybean oil/lecithin and their cytotoxicity on the HepG2 cell line. Food Chem 154, 282–290. https://doi.org/10.1016/j.foodchem.2014.01.012

Phan, Q.T., Le, M.H., Le, T.T.H., Tran, T.H.H., Xuan, P.N., Ha, P.T., 2016. Characteristics and cytotoxicity of folate-modified curcumin-loaded PLA-PEG micellar nano systems with various PLA:PEG ratios. Int J Pharm 507, 32–40. https://doi.org/10.1016/j.ijpharm.2016.05.003

Rosa, A., Nieddu, M., Pitzanti, G., Pireddu, R., Lai, F., Cardia, M.C., 2023. Impact of solid lipid nanoparticles on 3T3 fibroblasts viability and lipid profile: The effect of curcumin and resveratrol loading. J Appl Toxicol 43, 272–286. https://doi.org/10.1002/jat.4379

Storka, A., Vcelar, B., Klickovic, U., Gouya, G., Weisshaar, S., Aschauer, S., Helson, L., Wolzt, M., 2013. Effect of liposomal curcumin on red blood cells in vitro. Anticancer Res 33, 3629–3634.

In vivo

Busari, Z.A., Dauda, K.A., Morenikeji, O.A., Afolayan, F., Oyeyemi, O.T., Meena, J., Sahu, D., Panda, A.K., 2017. Antiplasmodial Activity and Toxicological Assessment of Curcumin PLGA-Encapsulated Nanoparticles. Front Pharmacol 8, 622. https://doi.org/10.3389/fphar.2017.00622

Dandekar, P.P., Jain, R., Patil, S., Dhumal, R., Tiwari, D., Sharma, S., Vanage, G., Patravale, V., 2010. Curcumin-loaded hydrogel nanoparticles: application in anti-malarial therapy and toxicological evaluation. J Pharm Sci 99, 4992–5010. https://doi.org/10.1002/jps.22191

Gopi, S., Jacob, J., Mathur, K.Y., 2016. Acute and subchronic oral toxicity studies of hydrogenated curcuminoid formulation “CuroWhite” in rats. Toxicol Rep 3, 817–825. https://doi.org/10.1016/j.toxrep.2016.10.007

Karabasz, A., Lachowicz, D., Karewicz, A., Mezyk-Kopec, R., Stalińska, K., Werner, E., Cierniak, A., Dyduch, G., Bereta, J., Bzowska, M., 2019. Analysis of toxicity and anticancer activity of micelles of sodium alginate-curcumin. Int J Nanomedicine 14, 7249–7262. https://doi.org/10.2147/IJN.S213942

Moshari, S., Nejati, V., Najafi, G., Razi, M., 2017. Nanomicelle curcumin-induced DNA fragmentation in testicular tissue; Correlation between mitochondria dependent apoptosis and failed PCNA-related hemostasis. Acta Histochem 119, 372–381. https://doi.org/10.1016/j.acthis.2017.03.007

Radmanesh, F., Razi, M., Shalizar-Jalali, A., 2021. Curcumin nano-micelle induced testicular toxicity in healthy rats; evidence for oxidative stress and failed homeostatic response by heat shock proteins 70-2a and 90. Biomed Pharmacother 142, 111945. https://doi.org/10.1016/j.biopha.2021.111945

Song, J.-W., Liu, Y.-S., Guo, Y.-R., Zhong, W.-X., Guo, Y.-P., Guo, L., 2022. Nano-Liposomes Double Loaded with Curcumin and Tetrandrine: Preparation, Characterization, Hepatotoxicity and Anti-Tumor Effects. Int J Mol Sci 23, 6858. https://doi.org/10.3390/ijms23126858

Thakkar, A., Chenreddy, S., Thio, A., Khamas, W., Wang, J., Prabhu, S., 2016. Preclinical systemic toxicity evaluation of chitosan-solid lipid nanoparticle-encapsulated aspirin and curcumin in combination with free sulforaphane in BALB/c mice. Int J Nanomedicine 11, 3265–3276. https://doi.org/10.2147/IJN.S106736

Tzankova, V., Gorinova, C., Kondeva-Burdina, M., Simeonova, R., Philipov, S., Konstantinov, S., Petrov, P., Galabov, D., Yoncheva, K., 2016. In vitro and in vivo toxicity evaluation of cationic PDMAEMA-PCL-PDMAEMA micelles as a carrier of curcumin. Food Chem Toxicol 97, 1–10. https://doi.org/10.1016/j.fct.2016.08.026

Yusuf, H., Novitasari, E.K.D.D., Purnami, N.L.W., Mahbub, A.W., Sari, R., Setyawan, D., 2022. Formulation Design and Cell Cytotoxicity of Curcumin-Loaded Liposomal Solid Gels for Anti-Hepatitis C Virus. Adv Pharmacol Pharm Sci 2022, 3336837. https://doi.org/10.1155/2022/3336837