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Data Article

Dataset of chicken-embryo blood cells exposed to quercetin, methyl methanesulfonate, or cadmium chloride

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ABSTRACT

Toxicological analysis of the effects of natural compounds is frequently mandated to assess their safety. In addition to more simple *in vitro* cellular systems, more complex biological systems can be used to evaluate toxicity. This dataset is comprised of bright-field microscopy images of chicken-embryo blood cells, a complex biological model that recapitulates several features found in human organisms, including circulation in blood stream and biodistribution to different organs. In the presented collection of blood smear images, cells were exposed to the flavonoid quercetin, and the two mutagens methyl methanesulfonate (MMS) and cadmium chloride ($CdCl_2$). *In ovo* models offer a unique opportunity to investigate the effects of various substances, pathogens, or cancer treatments on developing embryos, providing valuable insights into potential risks and therapeutic strategies. In toxicology, *in ovo* models allow for early detection of harmful compounds and their impact on embryonic development, aiding in the assessment of environmen-

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tal hazards. In immunology, these models offer a controlled system to explore the developing immune responses and the interaction between pathogens and host defenses. Additionally, *in ovo* models are instrumental in oncology research as they enable the study of tumor development and response to therapies in a dynamic, rapidly developing environment. Thus, these versatile models play a crucial role in advancing our understanding of complex biological processes and guiding the development of safer therapeutics and interventions. The data presented here can aid in understanding the potential toxic effects of these substances on hematopoiesis and the overall health of the developing organism. Moreover, the large dataset of blood smear images can serve as a resource for training machine learning algorithms to automatically detect and classify blood cells, provided that specific optimized conditions such as image magnification and background light are maintained for comparison. This can lead to the development of automated tools for blood cell analysis, which can be useful in research. Moreover, the data is amenable to the use as teaching and learning resource for histology and developmental biology.

Specifications Table

Subject	Health, Toxicology and Mutagenesis
Specific subject area	Blood cell image analysis, genotoxicity analysis, Hen's egg test for micronucleus induction (HET-MN), histology
Data format	Raw
Type of data	Image
Data collection	Ross 308 broiler strain were used as source of fertilized eggs. Quercetin was dissolved in DMSO and further dissolved in PBS. MMS and $CdCl_2$ were dissolved in 1 % DMSO in PBS. At day 8, the egg top was perforated to allow pipetting of each solution. At day 11, blood smears were performed, fixed and stained using Hemacolor kit protocol, and mounted using Entellan new. Slides were visualized by bright-field microscopy at 40 × magnification in a Nikon Eclipse E200 (Nikon) instrument. Images were captured with IC Capture 2.3 software, converted from TIFF to JPG and saved as 2592 × 1944 pixel files.
Data source location	Data was collected at the University of Porto (Department of Biology, Faculty of Sciences and Department of Chemical Sciences, Faculty of Pharmacy). The data is stored at University of Porto (Department of Chemical Sciences, Faculty of Pharmacy).
Data accessibility	Repository name: Mendeley Data Data identification number: 10.17632/5695ctj5cm.4 Direct URL to data: https://data.mendeley.com/datasets/5695ctj5cm/4

1. Value of the Data

- Data collection aimed to investigate effects of quercetin and mutagens on embryonic blood cell morphology, and the experiment was designed to assess toxicological and developmental impacts of these substances.
- Over 2,800 blood smear images were obtained from developing chicken embryos exposed to quercetin and mutagens. It represents a unique collection of bright-field images with diverse experimental conditions.

- The available images are of especial interest to researchers in toxicology, developmental biology, and drug development; educators and students in histology and biology; AI/ML researchers for image analysis applications.
- The data can be coupled for reuse with studies on toxicological effects of mutagens, hematopoiesis, and comparative analysis of blood cell development. In addition, there is potential application in automated blood cell analysis using machine learning.

2. Data Description

This dataset consists of microscopy images of blood smear samples from 59 chick embryos. Initially, PBS, $CdCl_2$ and MMS control were analyzed in one independent experiment (N = 1). Subsequently, MMS control was again used, together with DMSO control, and quercetin at various doses, in two independent experiments (N = 2). After the incubation time, blood smears were immediately prepared, dried, fixed, stained, and mounted for microscopy.

The microscopy image dataset consists of 2875 color TIFF images of 2592 × 1944 pixels at 24-bit depth, converted to JPG images of 2592 × 1944 pixels at 24-bit depth, distributed by the different experimental groups as described above and according to Table 1. The dataset images are included in the referenced data repository [1]. For more information on blood cell type, please refer to literature on standard classification of chicken embryo blood cells [2,3].

Table 1
Number of images for each blood smear, corresponding to one embryo.

	PBS	$CdCl_2$	MMS control			DMSO		Que 1 µg		Que 10 µg		Que 100 µg	
Embryo	N1	N1	N1*	N2**	N3	N1	N2	N1	N2***	N1	N2	N1	N2
1	20	26	60	60	22	59	60	60	60	60	59	60	60
2	21	24	60	–	24	60	60	60	60	60	57	60	60
3	25	20	–	–	27	60	60	60	60	60	69	60	60
4	20	23	–	–	32	60	60	60	59	15	60	43	60
5	23	30	–	–	26	60	60	60	–	60	59	64	60
6	21	27	–	–	–	–	–	–	–	–	–	–	–

* 2 eggs not viable; ** 3 eggs not viable; *** 1 egg not viable.

3. Experimental Design, Materials and Methods

Fertilized chicken eggs were obtained from the Ross 308 broiler strain. Embryo viability was confirmed by candling. Viable eggs were washed once with sterile distilled water and incubated at 38°C in a 70 %-humidified atmosphere. Eggs were positioned at 45° angle with pointed end facing downwards. During embryonic days (ED) 1 – 6, eggs were routinely rotated 90° around the longer axis, three times per day, to prevent attachment of blood vessels or embryo to the eggshell. The described incubation conditions were according to the literature regarding chicken egg incubation [4]. At ED7, the egg was repositioned with pointed end facing down and in full vertical position, to allow the centering of the air cell at the blunt end. Quercetin was initially dissolved in dimethyl sulfoxide (DMSO), as a stock solution, and further dissolved in phosphate-buffered saline (PBS). Cadmium chloride ($CdCl_2$, 133 µg/ml) and methyl methanesulfonate (MMS, 3.67 mg/ml) were dissolved directly in 1 % DMSO in PBS. The concentrations to use were based on previous experiments *in vitro* and *in ovo* [5–7]. All solutions were freshly prepared before inoculation. At ED 8, after removal of non-viable eggs, each egg top was perforated and covered with tape. Each egg was then inoculated by pipetting to the air cell, and immediately returned to the egg incubator. At ED 11, an opening was created on the top of each viable egg, using curved dissecting scissors. These times were based on previous toxicological assessments *in ovo*

[8,9]. PBS solution was then used to wet the inner membrane, which was locally removed with forceps. The largest blood vessel identified by visual inspection was then raised with the aid of a plastic strip. The vessel was rapidly washed with PBS, dried with paper tissue, and incised. Immediately, 200 µl of whole blood was pipetted from the strip to microscopy slide. Using another glass slide at 45°, blood smears were prepared and an alphanumerical code was attributed to each slide. Slides were left to dry at desiccator. After drying, blood smears were fixed with methanol and stained using Hemacolor kit (Sigma-Aldrich, St. Louis, MO-USA) protocol according to the manufacturer's instructions. Briefly, slides were quickly stained in eosin Y, followed by quickly staining in methylene blue/azure B mixture, and finally removing the excess stain in PBS. Slides were serially dehydrated in 70, 90, and 95 % ethanol solutions (v/v), and finally in absolute ethanol. The dried samples were mounted with Entellan new (Sigma-Aldrich, St Louis, MO-USA), and stored until visualization. The preparations were visualized at 40 × magnification by bright-field microscopy in a Nikon Eclipse E200 (Nikon) instrument. Images were captured with IC Capture 2.3 software, converted from TIFF to JPG and saved as 2592 × 1944 pixel files.

4. Limitations

Not all groups have the same number of embryo blood smears analyzed since eggs could become unviable until blood collection. In addition, after mounting of blood smears with Entellan, a small number of samples were not optimally preserved until microscopic visualization, resulting in blurred images.

Ethics Statement

The authors have read and followed the ethical requirements for publication in Data in Brief. From a regulatory point of view, chicken embryo experimentation is not considered to be animal testing, therefore no prior ethical reviews are mandated [10].

Data Availability

Effect of quercetin on the morphology of chicken embryo blood cells (Original data) (Mendeley Data).

CRediT Author Statement

José Miguel P. Ferreira de Oliveira: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition; **Lutete Daniel Lenda:** Methodology, Formal analysis, Investigation, Visualization; **Carina Proença:** Methodology, Investigation; **Eduarda Fernandes:** Resources, Writing – review & editing, Project administration, Funding acquisition; **Verónica Bastos:** Conceptualization, Formal analysis, Supervision; **Conceição Santos:** Conceptualization, Formal analysis, Resources, Writing – review & editing, Supervision.

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Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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