COLLECTION, PRODUCTION AND STORAGE OF BLOOD COMPONENTS

*Original article*

# *In vitro* exposure of whole blood to a cannabinoid mixture impairs the quality of red blood cells and platelets

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**Background** - In a recent study, 13.8% of blood donors had reported cannabis use in the 72 hours preceding their donation, and these donors are not deferred under existing criteria in Canada. This high prevalence raises concerns about the potential impact of cannabis use on the quality of blood products. The current study assessed the impact of a cannabinoid mixture on the quality of red blood cells and platelets, from the time of collection and processing to their storage.

**Materials and methods** - To mimic pre-donation cannabis use, whole blood was collected and exposed (in vitro) to varying concentrations (range: 1-24 µg/mL) of a cannabinoid mixture (CM) overnight. Whole blood was then separated into red blood cells (RBCs) and platelets-rich plasma (PRP), which were stored at 4°C (for RBCs) or at room temperature (for PRP). Flow cytometry analyses, hemolysis measurements and biochemical analyses were performed during the processing stage and throughout storage.

**Results** - In the RBC fraction, free hemoglobin levels were increased in a dose-dependent manner after the addition of a cannabinoid mixture to whole blood. Hemolysis and methemoglobin levels were significantly higher in CM-exposed RBCs than CM-free controls, after processing and throughout storage. Furthermore, platelet counts and CD62P expression (on day 7 post-separation) were significantly lower in CM-exposed PRP than cannabinoid-free PRP controls. The aggregation potential of CM-exposed platelets was significantly lower than that of cannabinoid-free controls, after the processing and throughout storage.

**Discussion** - An *in vitro* exposure to a cannabinoid mixture hemolyzed RBCs, impaired oxygen transport by RBCs, reduced platelet counts, and impaired platelet function. These results suggest that pre-donation cannabis use might impair the quality of blood products.

**Keywords:** *cannabinoids, platelets, red blood cells, hemolysis, aggregation capacity.*

## INTRODUCTION

Cannabis has long been used as a medical treatment, whether it be as a painkiller, anti-inflammatory agent, anticonvulsant agent, or other uses. It contains more than 60 chemical compounds ("cannabinoids"), but the two main ones are $\Delta^9$-tetrahydrocannabinol (THC) and cannabidiol (CBD)[1]. When cannabis is smoked, THC and other plant compounds

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enter the blood circulation and are rapidly carried to the brain[2,3]. Cannabinoids exert their effect primarily by binding to the cannabinoid 1 (CB1) and cannabinoid 2 (CB2) receptors which are expressed in the brain as well as on immune cells and platelets[4].

Canada legalized the recreational use of cannabis in 2018[5,6], but deferral criteria for blood donation have not been updated since then. Specifically, deferral only applies to donors who are intoxicated and thus unable to understand screening questions and provide informed consent. In a recent survey study, 32.6% of blood donors reported trying cannabis at least once, 40.3% reported using cannabis (edibles, smoke and/or vape) less than once-monthly with a minority reporting using it daily (6.8%) or few times a week (5.8%). Importantly, for donors who reported marijuana use, 13.8% of respondents stated they have used cannabis within 72 h before giving blood[7]. This high prevalence of pre-donation cannabis use among blood donors raises questions about its potential impact on the quality of blood products.

The effect of cannabinoids on the quality of blood products is not completely understood, with some conflicting evidence. With regard to platelets, Deusch *et al.* showed that adding THC (0.319 µg/mL-31.9 µg/mL) to whole blood increases the expression of glycoprotein IIb/IIIa and P-selectin, suggesting THC activates platelets and exerts a pro-coagulant effect[8]. Conversely, another study has shown that various cannabinoids (including THC and CBD) hinder the aggregation of isolated platelets, suggesting cannabinoids exert an anticoagulant effect[9]. With regard to red blood cells (RBCs), some studies have reported reduced RBC counts among cannabis users, possibly due to eryptosis[10,11].

Therefore, we evaluated the effect of an *in vitro* exposure of whole blood to a cannabinoid mixture, from the time of processing and throughout storage. We show that such brief exposure hemolyzes RBCs, impairs platelet function, and worsens many other blood quality parameters.

## MATERIALS AND METHODS

### Informed consent and blood collection

This study was approved by Héma-Québec's Research Ethics Committee (CER#2020-010), and all participants signed an informed consent form. Whole blood (450 mL) was collected using the Leukotrap® WB system (Haemonetics, Braintree, MA, USA) according to the manufacturer's instructions.

### Cannabinoid exposure

Aliquots of 1.950 mL of anticoagulated whole blood were split among the wells of a 24-well microplate (Thermo Fisher Scientific, Waltham, MA, USA). Samples in individual wells were exposed to the following conditions for ≥12 hours at 37°C/5% $CO_2$: (1) cannabinoid mixture-8 components dissolved in methanol (CM; at 1, 6 or 24 µg/mL; C-219, Cerilliant, Round Rock, Tx, USA), (2) whole blood + volume of methanol 90% (0.18%; 1.08% and 4,8%; i.e., vehicle), or (3) whole blood alone (i.e., untreated control [UT]).

Moreover, three 150 mL DEHP-PVC bags (Fenwal, Lake Zurich, IL, USA) containing 40 mL of whole blood were supplemented with 1.920 mL of a cannabinoid mixture (CM) for a final concentration of 24 µg/mL, 1.920 mL of methanol (i.e., vehicle), or nothing (i.e., untreated control). All three bags were agitated (80 rpm) overnight on a stirrer plate (VWR® Advanced Dura-Shaker for Extreme Environments; #10159-960) for ≥12 hours at 37°C/5% $CO_2$.

### Evaluation of the residual cannabinoid concentration

For experiments carried out in bags, the concentration of residual plasma cannabinoids was measured using the Cannabis Analyzer Model LC2030C Plus (Shimadzu, Columbia, MD, USA) at the Syneos Health™ facility in Québec City. Briefly, each plasma sample was centrifuged at 112 × *g* for 2 min at room temperature (RT). Then, 200 µL of the supernatant was mixed with 4.8 mL of methanol and centrifuged again. The supernatant was collected and analyzed by high-performance liquid chromatography-ultraviolet. The concentrations of each cannabinoid in the mixture (eight in total) were determined based on a standard calibration curve that ranged from 0.125 to 62.5 µg/mL.

### Blood separation, storage and sampling

Whole blood exposed to the above-mentioned conditions was then separated into RBCs and platelet-rich plasma (PRP) by centrifugation at 400 × *g* for 10 min, without brake. For the PRP fraction, 5 mL of each experimental condition was transferred into a pooling platelet bags (three quarters of the bag was sealed using a tabletop sealer to allow a 5 mL volume of PRP) removed from

platelet pooling set (Reveos®; Terumo, Elkton, MD, USA; #41910) and agitated at 30 rpm using a stirrer plate (VWR Signature™ Rocking Platform Shaker #12620906) at RT for 7 days. To avoid resampling the same bag, two bags of each condition were generated and used to measure biochemical factors (ATP, sodium, potassium, lactate and pH) and to perform flow cytometry analyses immediately after separation, before storage (day 0), on day 1 post-component separation, and on day 7 post-component separation. More precisely, for each bag, 1 mL was used for whole blood count, 500 µL used for ATP analysis, 500 µL used for the analysis of biochemical factors other than ATP, 110 µL used for flow cytometry analysis and platelet count and 2 mL for aggregation analysis. Storage time were chosen based on standards council of Canada policy to which Hema-Québec adheres and which permit platelets storage until day 7[12]. For the RBCs, solution saline-adenine-dextrose-mannitol (SAG-M) was added to a ratio 1: 2 after leukoreduction using an Acrodisc® WBC 25 mm PSF filters (Pall Laboratory, Port Washington, NY, USA). Then, 5 mL of RBCs were transferred into four storage bags (40 mL sampling pouch [VSE0000Y], Macopharma; UK Ltd, Twickenham, UK), which were stored at 4°C for 42 days. For each bag, 1 mL was used for whole blood count, 500 µL used for ATP analysis, 1.5 mL was used for hemolysis, methemoglobin and other biochemical factors analysis (sodium, potassium, lactate and pH) were measured on days 0, 1, 7, 21 and 42 post-separation (**Figure 1**). Storage time were chosen based on standard council of Canada policy to which

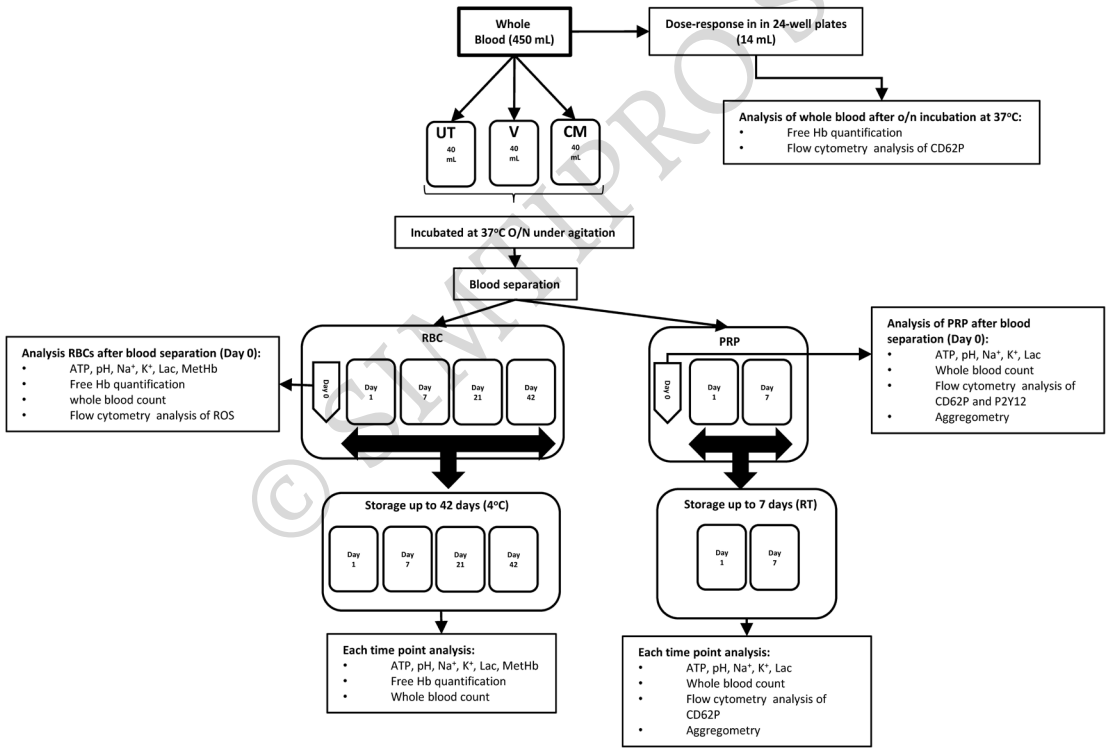


**Figure 1 - Schematic representation of the protocol**
450 mL of whole blood was collected using the Leukotrap® WB system according to the manufacturer's instructions. In the first set of experiments 14 mL was used for a dose-response analysis of CM while, the remaining amount of whole blood was used in another research project. In a second set of experiments, 120 mL (40 mL x 3) out of the 450 mL collected were transferred in three 150 mL DEHP-PVC bags, CM and methanol were added in the corresponding bags and incubated overnight at 37°C/5% $CO_2$. Whole blood exposed to CM, to methanol and untreated were then separated into RBCs and platelet-rich plasma (PRP) by centrifugation. For the PRP fraction, 5 mL of each experimental condition was transferred into a pooling platelet bags and stored at RT for 7 days. Analysis of biochemical factors and flow cytometry analyses were performed before storage (day 0), on day 1 of storage and on day 7 of storage. For the RBCs, after leukoreduction, 5 mL of RBCs were transferred into four storage bags which were stored at 4°C for 42 days. For each bag, hemolysis, methemoglobin and other biochemical factors analysis (sodium, potassium, lactate and pH) were measured on days 0, 1, 7, 21 and 42 of storage.



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Hema-Québec adheres and which permit storage of RBCs until 42 days after separation[12]. However, all-time points between day 1 and day 42 were randomly chosen in order to have a better view of what happens in the bags during storage.

### Flow cytometry

Throughout the study, platelets were enumerated by flow cytometry. Briefly, PRP was diluted 1/160 in DPBS 1X, incubated 15 min with FITC-Mouse Anti-Human CD41a (BD Biosciences, Franklin Lakes, NJ, USA), and analyzed with the BD Accuri™ C6 flow cytometer (BD Biosciences). In addition, platelet activation was assessed by measuring the expression of CD62P using a PE-conjugated mouse anti-human CD62P antibody (Beckman Coulter, Brea, CA, USA) according to the manufacturer's instructions. We also looked at the expression of P2Y12, a receptor involved in platelets aggregation[13], using a PE-conjugated mouse anti-human P2RY12 antibody (BioLegend, San Diego, CA, USA). Briefly, platelets were incubated 10 min with FcR blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany), in the dark, at 4°C followed by 20 min in Cell Staining Buffer (BioLegend) at RT. Platelets were then washed with Cell Staining Buffer, centrifuged 5 min at 350 × *g*, and diluted in the same buffer for analysis. All data were acquired with the BD Accuri™ C6 flow cytometer (BD Biosciences). Total oxidative stress in RBCs was evaluated using the intracellular total ROS assay kit (ImmunoChemistry technologies, Davis, CA, USA), according to the manufacturer's instructions. All flow cytometry data were analyzed using the FCS Express™ 6 software (De Novo Software, Los Angeles, CA, USA).

### Hemolysis

Free hemoglobin (free Hb) in plasma and in RBC supernatants was measured using the HemoCue®Plasma/Low Hb System (HemoCue, Brea, CA, USA). Complete blood counts were performed with a Coulter Ac•T 5diff AL hematology counter (Beckman Coulter, Miami, FL, USA). The percentage of hemolysis was calculated as follows, based on the concentrations of free Hb and hematocrit (Ht):

*Hemolysis %= free Hb (g/dL) * 10 * (100- [Ht (L/L) * 100])/Hb total (g/L)*

### Biochemical factors and hematology analysis

The biochemical factors of interest included sodium (Na+), potassium (K+), lactate, pH and methemoglobin (MetHb) levels. These factors were assessed with the point of care blood gas analyzer ABL90 FLEX PLUS (Radiometer, Copenhagen, Denmark). Adenosine triphosphate (ATP levels were assessed using the ATP lite Luminescence Assay (PerkinElmer, Waltham, MA, USA), according to the manufacturer's instructions.

### Platelets aggregation

Two millilitres (2 mL) of PRP was collected to perform aggregation analysis. 1 mL out of 2 was used to generate Poor Platelet Plasma (PPP). This latter was used as a 100% transmittance (PPP cuvette). 500µL of PRP was then placed in an aggregometer cuvette (Platelet Ionized Calcium Aggregometer, Chrono-Log). Samples were stirred at 1200 rpm and activated by the addition of 20 mM of PPACK-Dihydrochloride (#142036-63-3 - Calbiochem [EMD Chemicals, San Diego, CA, USA]), 0.2 M of CaCl2 (Sigma-Aldrich, St. Louis, Missouri, USA) and 10 µM of ADP (Sigma-Aldrich) and allowed to run for 6 min. The aggregometer was calibrated by setting PRP as 0% aggregated and PPP as 100% aggregated.

### Statistical analysis

All analyses were performed using GraphPad Prism 9.2.0 (GraphPad, San Diego, CA, USA). Values are presented as mean ± standard error of the mean (SEM) and statistical analyzes were carried out using a Kruskal-Wallis test (nonparametric ANOVA), Wilcoxon matched pairs signed-ranks test and a Mann-Whitney test where applicable. A p-value below 0.05 was considered statistically significant.

## RESULTS

### Exposure of whole blood to a cannabinoid mixture significantly increases free hemoglobin levels but does not affect platelet activation

Cannabinoids are detectable in the plasma within a few seconds after the first inhalation, and their concentration peak within 3-10 min[14,15] and bioavailability following the smoking route was reported as 2-56%, due in part to intra- and inter-subject variability in smoking dynamics, which contributes to uncertainty in dose delivery[16,17]. In Quebec, the cannabinoid concentrations in cannabis sold at *Société Quebecoise du Cannabis* (SQDC; the only authorized distributor in the province) vary between 1 mg and over 300 mg/g of cannabis. We decided to test a scenario corresponding to the use of 1 g of cannabis containing 240 mg of cannabinoids, i.e., 24%, which is

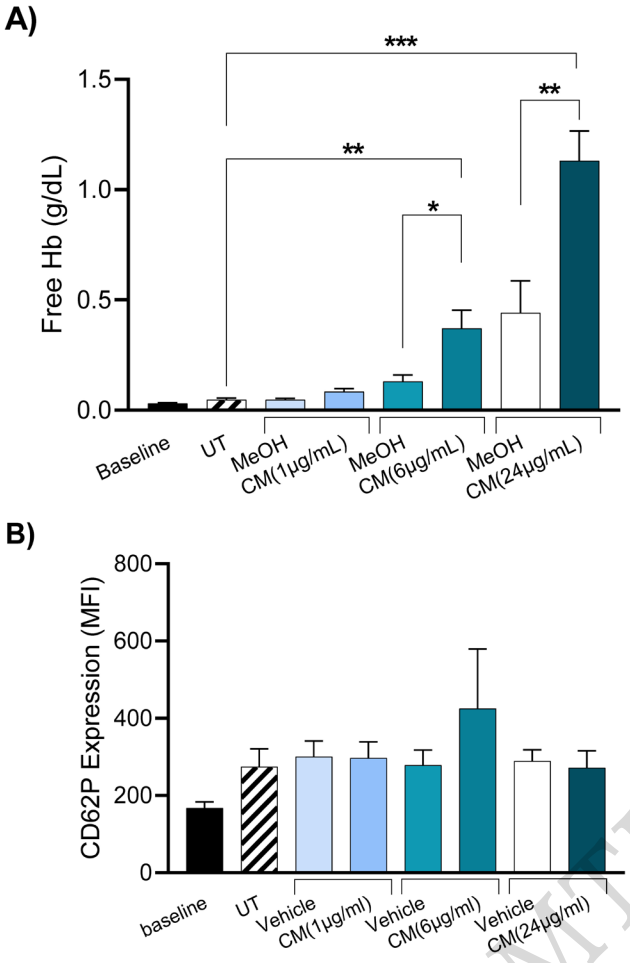


**Figure 2 - Effect of an exposure to a cannabinoid mixture on free hemoglobin (free Hb) and CD62P expression in whole blood**

**(A)** Free Hb levels after an overnight exposure of whole blood to a cannabinoid mixture or the corresponding volume of methanol (i.e., vehicle control; n=5). **(B)** CD62P expression on platelets after exposure to varying concentrations of the cannabinoid mixture or the corresponding volume of methanol (i.e., vehicle control; n=5). Means ± standard errors of the mean are represented. *: p<0.05, **: p<0.001, ***: p<0.0001.

in the range of commercially available products. Thus, in order to determine the dose of cannabinoids to be used for experiments in bags, we performed a dose response in a 24-well plates. So, exposing whole blood to a CM increased free Hb levels in a dose-dependent manner. Relative to untreated controls, free Hb levels were 1.75 folds higher with a 1 µg/mL cannabinoid mixture, 7.75 folds higher with a 6 µg/mL mixture, and 23.5 folds higher with a 24 µg/mL mixture (**Figure 2A**). Free Hb levels were also higher in methanol-exposed samples, but only with volumes corresponding to those in the 6 or 24 µg/mL of CM. Further, the effect of methanol was less pronounced than that of the CM (~2 folds smaller increase; **Figure 2A**). In addition, the expression of CD62P (a marker of platelet activation) did not significantly change following treatment with CM or methanol, regardless of the concentration tested (**Figure 2B**).

We also assessed the residual concentration of cannabinoids in our *in vitro* experimental model. A bag containing 40 mL of whole blood was treated with a 24 µg/mL of CM and incubated overnight at 37°C. Following treatment and processing (on day 0), plasma samples were recovered and residual cannabinoid levels were measured. As expected, the concentration of cannabinoids was significantly lower (~10 folds) than after the initial spike and was not detectable in untreated samples (**Table I**), thereby validating our experimental model.

## Exposure to a cannabinoid mixture triggers hemolysis and impairs the function of red blood cells

Following overnight exposure to a 24 µg/mL of CM, whole blood was separated into RBCs and PRP (**Figure 1**). One bag of RBCs from each experimental condition was used to assess hemolysis and the level of biochemical factors. CM-exposed RBCs exhibited ~2 times higher levels of free Hb and hemolysis compared with methanol-exposed RBCs,

**Table I** - *Residual plasma concentration of the cannabinoid mixture*

| | Cannabinoid concentration (µg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **CBDV** | **THCV** | **CBD** | **CBG** | **CBN** | **$\Delta^9$-THC** | **$\Delta^8$-THC** | **CBC** |
| **CM (spike)** | 24 (100%) | 24 (100%) | 24 (100%) | 24 (100%) | 24 (100%) | 24 (100%) | 24 (100%) | 24 (100%) |
| **Untreated (Day 0)** | ND | ND | ND | ND | ND | ND | ND | ND |
| **CM (Day 0)** | 2.3 (9.6%) | 2.83 (11.8%) | 1.96 (8.7%) | 1.75 (7.3%) | 2.37 (9.8%) | 2.84 (11.8%) | 3.04 (12.6%) | 3.52 (14.6%) |

CBC: cannabichromene; CBD: cannabidiol; CBDV: cannabidivarin; CBG: cannabigerol; CBN: cannabinol; THC: tetrahydrocannabinol; THCV: tetrahydrocannabivarin; ND: not detectable.



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for all-time points tested (**Figure 3A-B**). ATP levels decreased in a similar way for all conditions tested throughout storage (**Figure 3C**). Sodium levels were relatively stable throughout storage and did not significantly differ across conditions (**Figure 3D**). By contrast, potassium and lactate levels increased and were higher in CM-exposed samples than untreated or methanol-exposed samples, although lactate levels were similar across conditions on day 42 (**Figure 3E-F**). The pH gradually declined throughout storage and was consistently lower (i.e., more acid) in CM-exposed samples than untreated or methanol-exposed samples (**Figure 3G**). Furthermore, the percentage of MetHb was consistently higher (~1.5 folds) in CM-exposed RBCs compared with untreated

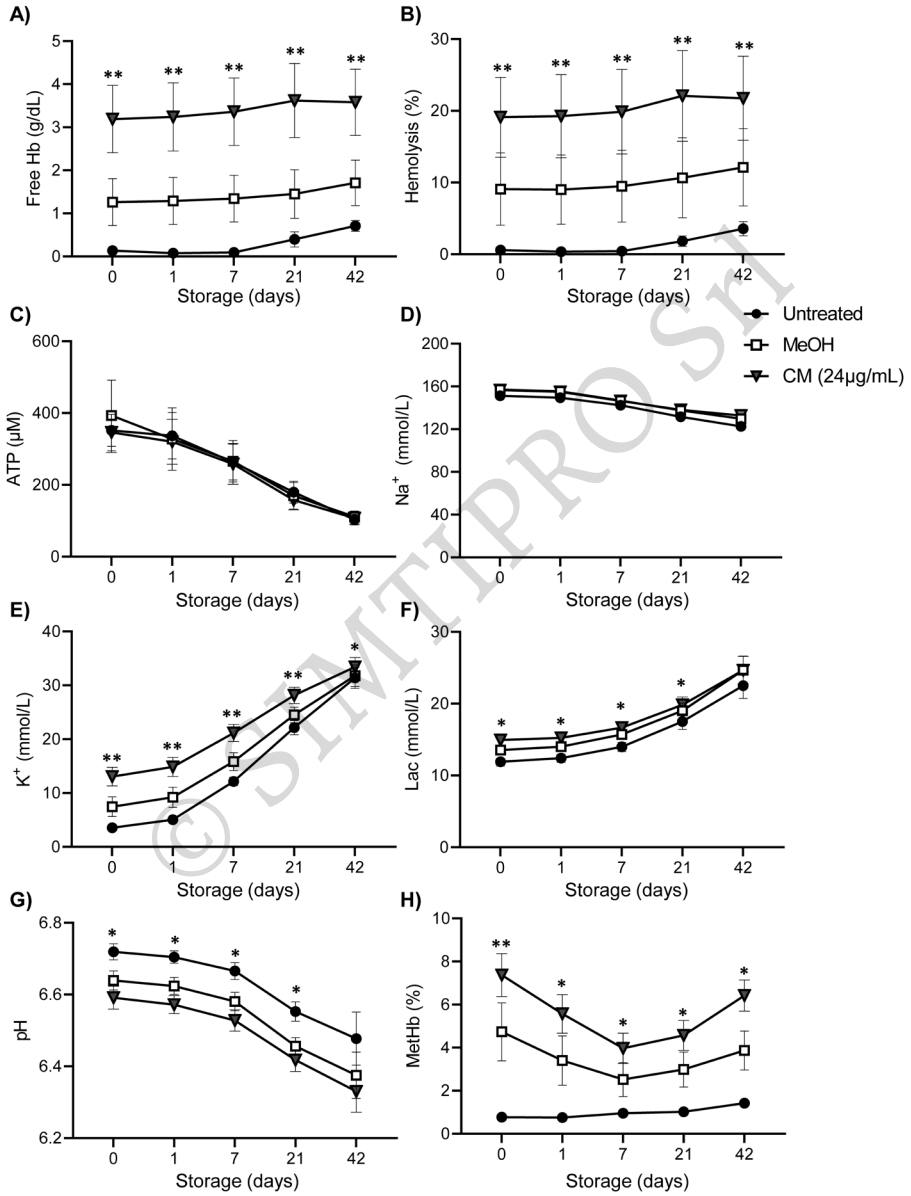




**Figure 3 -Effect of exposing RBCs to a cannabinoid mixture**
**(A)** Free Hb levels and the percentage of RBCs hemolysis following exposure of whole blood to a 24 µg/mL cannabinoid mixture, after processing and during storage; **(B)** Percentage of RBCs hemolysis following exposure of whole blood to a 24 µg/mL cannabinoid mixture, after processing and during storage; **(C)** ATP levels in RBCs following the exposure of whole blood to a 24 µg/mL cannabinoid mixture, after processing and during storage; **(D)** Biochemical analysis of sodium ($Na^+$); **(E)** potassium ($K^+$); **(F)** lactate; **(G)** pH; **(H)** and methemoglobin (MetHb) in RBCs following the exposure of whole blood to a 24 µg/mL cannabinoid mixture, after processing and during storage (n=6). Means ± standard errors of the mean are represented. *: p<0.05, **: p<0.001.

or methanol-exposed samples throughout storage (**Figure 3H**).

### Exposure to a cannabinoid mixture impairs the activation and aggregation capacity of platelets

PRP samples were collected on days 0, 1, and 7 to assess platelet counts and activation. Throughout storage, platelet counts were consistently lower in CM-exposed bags than untreated or methanol-exposed bags (**Figure 4A**). Similar to what was observed with whole blood, CM-exposed platelets had similar proportions of CD62P-positive cells at days 0 and 1, but not on day 7 when platelets CD62P expression significantly increased in untreated samples and remained stable in CM- and methanol-exposed samples (**Figure 4B**). ATP levels (i.e., a marker of platelet function) were significantly lower for CM-exposed samples than untreated or

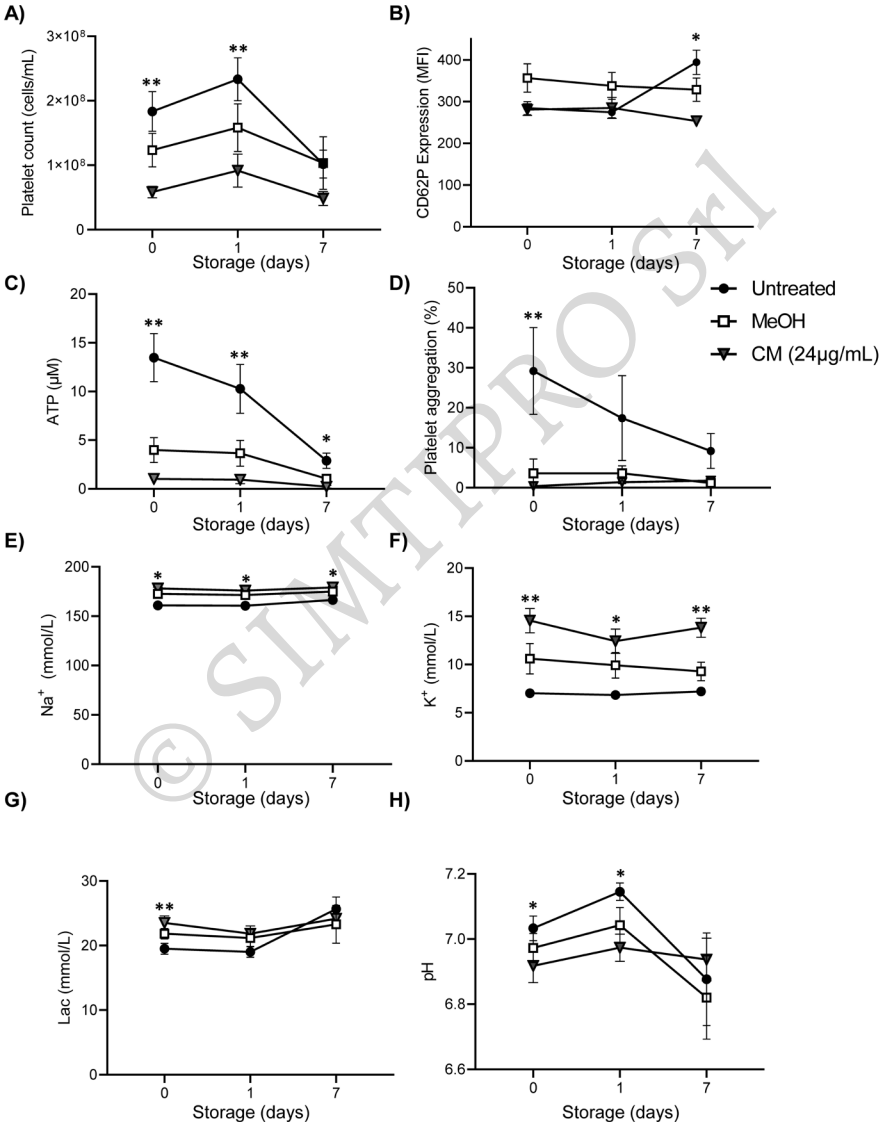




**Figure 4 - Effect of exposing PRP to a cannabinoid mixture**

**(A)** Flow cytometry counts of CD41a-positive platelets following whole blood exposure to a 24 µg/mL cannabinoid mixture, after processing and during storage; **(B)** Expression of CD62P on platelets following whole blood exposure to a 24 µg/mL cannabinoid exposure, after processing and during storage; **(C)** Aggregation capacity assessed following whole blood exposure to 24 µg/mL, after processing and during storage; **(D)** ATP levels in platelets following whole blood exposure to a 24 µg/mL cannabinoid mixture, after processing and during storage; **(E)** Biochemical analysis of sodium ($Na^+$); **(F)** potassium ($K^+$); **(G)** lactate and **(H)** pH measured in PRP bags following whole blood exposure to a 24 µg/mL cannabinoid mixture, after processing and during storage (n=6). Means and standard errors of the mean are represented. *: p<0.05, **: p<0.001.



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methanol-exposed samples at all time points tested (**Figure 4C**). In addition, platelet aggregation was substantially reduced in CM-exposed samples, although this decrease was only statistically significant on day 0 (**Figure 4D**). Sodium and potassium levels were significantly higher for CM-exposed samples than untreated or methanol-exposed samples at all time points tested but no difference was observed for all conditions tested throughout storage (**Figure 4E-F**). Lactate levels were significantly higher in CM-exposed platelets than untreated or methanol-exposed platelets on day 0, but no statistically significant difference was observed on day 1 and day 7 (**Figure 4G**). The pH of CM-exposed PRP was significantly lower than untreated and methanol-exposed PRP on days 0 and 1, but not on day 7 (**Figure 4H**).

### Exposure to a cannabinoid mixture increases the oxidative stress in red blood cells and reduces the expression of P2Y12 receptor on platelets

Reactive oxygen species (ROS) damage RBC membrane and contribute to RBCs hemolysis[18,19]. We assessed intracellular ROS levels in RBCs (**Figure 5A**) following exposure to a cannabinoid mixture (at day 0) and observed a significant increase, which was correlated to an increased level of free Hb **(Figure 3A)**.

To explore how platelet function was affected by cannabinoid exposure, we assessed the cell-surface expression of P2Y12, a Gi-coupled ADP receptor that plays a central role in platelets function[13]. The expression of P2Y12 was significantly reduced on CM-exposed platelets compared with untreated platelets or methanol-exposed platelets, supporting a potential role of P2Y12 in platelets reduced functionality induced by CM (**Figure 5B**).

## DISCUSSION

In many countries (including Canada), current regulations do not require blood banks to inquire donors about cannabis use. The donor's capacity to answer screening questions and provide informed consent is the main factor that determines eligibility. While cannabinoids might affect blood cells[20-22] and platelets' responsiveness to different stimuli[8,9], no evidence currently suggests that pre-donation exposure to cannabinoids impairs the quality of the blood products. Here, we report that exposing (*in vitro*) whole blood to cannabinoids significantly increases free Hb levels, triggers hemolysis in RBC units, and reduces platelet aggregation capacity in PRP.

Cannabinoids are detectable in the plasma within a few seconds after the first inhalation, and their concentration peaks within 3-10 min[14,15]. They are quickly eliminated by pyrolysis and their bioavailability following the smoking route was reported as 2-56%, due in part to intra- and inter-subject variability in smoking dynamics,

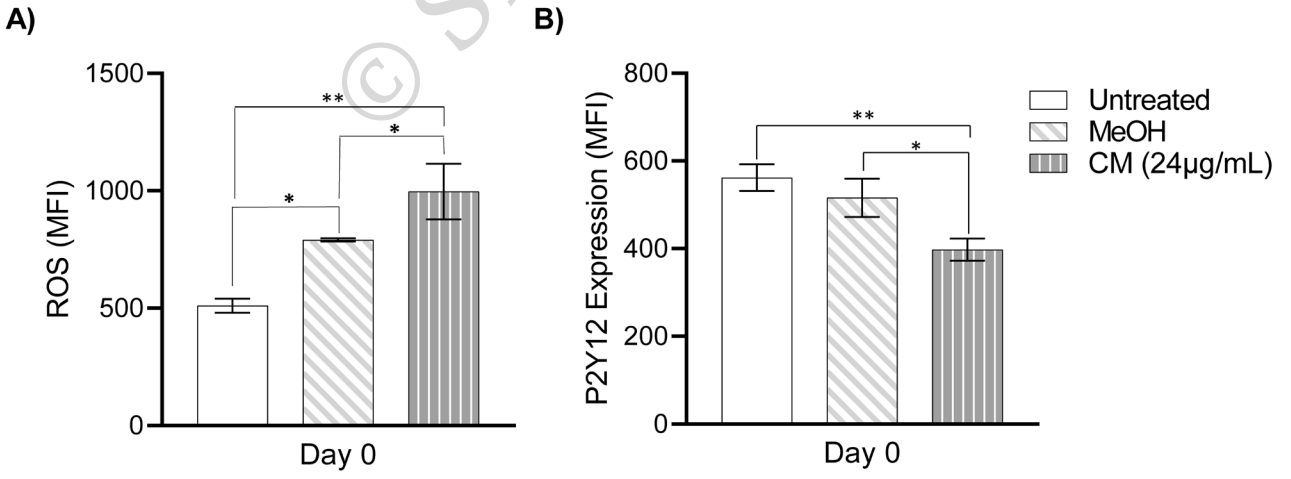


**Figure 5 - Effect of exposing whole blood to a cannabinoid mixture on reactive oxygen species (ROS) in RBCs and P2Y12 receptor expression on platelets**

**(A)** Median fluorescence intensity of total intracellular ROS in RBCs, with or without exposure to a 24 µg/mL cannabinoid mixture (n=5); **(B)** Median fluorescence intensity of P2Y12 on platelets following exposition or not to 24 µg/mL of CM measured by flow cytometry (n=5). Data are presented as means and standard errors of the mean are represented. *: p<0.05, **: p<0.001

which contributes to uncertainty in dose delivery[16,17]. For example, it has been reported that six min after the inhalation of 13 mg of THC ($2.5\times10^{19}$ molecules) only 2.8% of this THC ($1.4\times10^{14}$molecules/mL) was detected in the plasma[16]. Moreover, Agurell et *al.*[23] have reported that one minute after IV administration of a single bolus of 5 mg THC ($9.55\times10^{18}$ molecules) a plasma concentration of $4.28\times10^{14}$ molecules/mL was observed, supporting a rapid elimination of cannabinoids but above all, a difference between the dose that comes into contact with blood and the one measured in it. We thus selected the doses considering a bioavailability of 2% (1 µg/mL), 12,5% (6 µg/mL) and 50% (24 µg/mL) equivalent to a 1 g joint of cannabis containing 24% of cannabinoids and performed a dose-response to determine which concentration to use for experiments performed in bags. Thus, a dose-response in 24-well plates using CM revealed the ability of the latter to induce a significant and dose-dependent increase of the free hemoglobin level (**Figure 2A**). The fact that most cannabinoids had their concentration reduced by ≤90% on day 1 –as anticipated based on previous observations in cannabis users[2,3]– further supports the validity of our experimental model.

Based on this finding, we decided to separate the whole blood into blood components namely RBCs and PRP. Cannabinoids had a significant effect on hemolysis, as measured by free Hb levels (**Figure 3A** and **3B**). In untreated samples, hemolysis levels were in the normal range (i.e., <0.8%)[24] from days 0 to 7, and increased to 1% at day 21 and to 3% at day 42. This increase might be due to different parameters including premature aging induced by the incubation at 37°C, the use of a small sample bag in lieu of a regular storage bag (due to the small volume transferred in the bag) and by the use of manual leukoreduction protocol. By contrast, methanol-exposed RBCs had a mean hemolysis levels of 1.5%, and cannabinoid-exposed RBCs had ≥9.8% of hemolysis up until day 42 and would thus have been discarded. The methanol-induced hemolysis levels was unexpected, as Sonmez et *al.* reported that several alcohols –but not methanol– trigger hemolysis[25]. This discordance may be explained by the higher final concentration of methanol (~4.8%) used in our experiments. However, the hemolysis induced by methanol was approximately half as pronounced as that induced by cannabinoids, confirming a cannabinoid-specific effect on hemolysis. This increase of hemolysis is in accordance with a recent observation made by Kang DG et *al.*[26] who showed a strong hemolysis ability of cannabinoids that could be mediated by cell shrinkage and cell membrane scrambling as observed with anandamide[10] (a structural analog of THC), but it remains unclear whether a similar mechanism underlies our observations. Unexpectedly, the hemolysis levels of cannabinoid-exposed and methanol-exposed samples did not increase throughout storage, possibly because cell damages are already so high that the fluctuations are no longer significantly detected. This hypothesis is supported by the constant increase of potassium, which is strongly correlated to hemolysis[27,28], in all conditions. Abnormally high potassium levels can increase recipients' risk of cardiac arrhythmia, particularly for neonatal or infant recipients receiving large blood volumes[27-30]. The environment of cannabinoid-exposed samples also became more acidic, and these samples exhibited higher lactate levels. A cannabinoid-specific increase in MetHb levels was also observed, which suggested cannabinoids increase the levels of endogenous ROS[31]. Consistent with this hypothesis, ROS levels were significantly higher in cannabinoid-exposed samples than methanol-exposed samples at day 0. These un-neutralized ROS may damage the membrane of RBCs and thus impair the transport of oxygen to tissues[32,33].

Platelet counts were consistently lower in cannabinoids condition than untreated or methanol-exposed condition suggesting a potential ability of cannabinoid to induce spontaneous aggregation of the latter in a way similar to that observed with cold[34]. The expression of CD62P, a marker of platelet activation, was not affected on platelets isolated from whole blood by an *in vitro* exposure to cannabinoids. This result was surprising, as the opposite was observed by Deusch et *al.*[8]. This apparent discrepancy could be because we used a mixture of eight cannabinoids instead of THC alone, like Deusch *et al.*[8]. However, the expression of CD62P was significantly lower in the PRP fraction of cannabinoid-exposed or untreated samples relative to methanol-exposed samples at days 0 and 1. Further, CD62P expression remained stable at day 7 in cannabinoid-exposed and methanol-exposed samples, whereas it significantly increased in untreated samples. Cannabinoids therefore appear to prevent platelet

activation, which might permit extended platelet storage. These results are in line with a study by Zhuang *et al.*[35] in which the addition of anandamide to platelets significantly lowered soluble P-selectin and enable extended storage.

Despite this reduced activation of platelets, exposure to cannabinoids also significantly reduced platelet aggregation capacity, as shown by the reduction of ATP levels and P2Y12 receptor expression. Extracellular ATP regulates platelet reactivity either directly through its action on platelet purinergic receptors or indirectly through AMP (after hydrolysis), which inhibits platelets aggregation[36]. This claim was also supported by the reduced aggregation response of platelets following stimulation with ADP. Methanol alone significantly reduced ATP levels and aggregation response and had a modest effect on P2Y12 receptor expression, consistent with published data that high concentrations of methanol prevent platelet aggregation[37]. However, the magnitude of the reduction observed with cannabinoid exposure was greater than that observed with methanol alone, consistent with a cannabinoid-specific inhibitory effect previously reported by Formukong *et al.*[9] but a synergistic effect of MeOH and CM cannot be ruled out. This effect is further supported by the fact that cannabinoids increased the levels of potassium, whose dietary supplementation was reported to diminish platelets aggregation[38].

## CONCLUSIONS

To our knowledge, this study is the first evidence that briefly exposing (*in vitro*) whole blood to cannabinoids impairs the quality of blood products. Specifically, cannabinoids triggered RBCs hemolysis and impaired the capacity of platelets to aggregate, despite their rapid elimination after being spiked to whole blood. These impairments persisted following separation of whole blood into RBCs and PRP and throughout storage, as routinely carried out by blood banks. Although our *in vitro* model likely reproduces several features of cannabis use, synergistic effects of methanol and the cannabinoid mixture cannot be ruled out. So, further studies conducted among blood donors who use cannabis are required to confirm and to better understand the impact of cannabis use on the quality of blood products.

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## AUTHORSHIP CONTRIBUTIONS

Conceptualization: LL. Investigation and validation: MCL, and CDT. Writing: MCL and LL.

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