Continuous Blood Exchange in Rats: A Protocol for Experimental Investigation

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Abstract

Blood exchange therapy, specifically whole blood exchange (WBE), is increasingly being utilized in clinical settings to effectively treat a range of diseases. Consequently, there is an urgent requirement to establish convenient and clinically applicable animal models that can facilitate the exploration of blood exchange therapy mechanisms. Our study conducted continuous whole blood exchange (WBE) in rats through femoral and tail vein catheterization using dual-directional syringe pumps. To demonstrate the applicability of continuous whole blood exchange, drug-induced hemolytic anemia (DIHA) was induced through phenylhydrazine hydrochloride injection. Notably, the rats of WBE treatment group survived all and recovered within the subsequent period. After the implementation of continuous whole blood exchange therapy day (Day 1), the treatment group exhibited a statistically significant increase in red blood cells (P=0.0343) and hemoglobin levels (P=0.0090). The rats in the WBE treatment group exhibited a faster recovery rate compared to the model group, indicating the successful establishment of a continuous blood exchange protocol. This experimental approach demonstrates not just promising efficacy in the treatment of DIHA and offers a valuable tool for investigating the underlying mechanisms of blood exchange. Furthermore, it has a great potential to the advancement of biomedical research such as drug delivery exploration.
1 Introduction

Currently, blood exchange therapy, including whole blood exchange (WBE), are gradually being widely applied in clinical practice for the treatment of various diseases with good therapeutic outcomes\cite{1-4}. Blood exchange procedures have played a crucial role in clinical practice for the treatment of various medical conditions. Based on the modified blood exchange technique developed by Professor Li BJ from our transfusion department, not only has this method achieved favorable clinical outcomes, but it has also been documented in numerous clinical articles\cite{11}. A real-world study exploring the efficacy of whole blood exchange in SLE-AIHA patients showed that this treatment may serve as a safe and beneficial alternative for refractory severe SLE-AIHA\cite{3}. Another study indicated that lymphoplasmapheresis, a novel blood exchange combining conventional plasma exchange and lymphocyte removal\cite{5, 6}, required less plasma and was significantly effective in treating patients with severe myasthenia gravis\cite{5}. Despite the good clinical efficacy of blood exchange include plasma exchange (PE), its therapeutic mechanism remains unclear\cite{7, 8}. Therefore, there is a pressing need to establish convenient and clinically relevant animal models for exploring the mechanisms of blood exchange therapy, in order to facilitate our understanding of this treatment modality.

Blood exchange models are experimental methods that involve the transfer of blood or blood components between two animals, which are commonly used to investigate the effects of certain substances or treatments on the blood and other bodily systems\cite{8-14}. Previous study show that those models have been used for a variety of purposes, including the study of blood disorders\cite{9, 10, 15}, the investigation of immune system function\cite{16}, and the testing of medical therapies\cite{17-19} and etc. Due to their similar anatomical and physiological characteristics to humans, large animals such as lambs, dogs, and pigs were used in blood exchange models\cite{10, 12, 20, 21}. Those large animals are used in complex and discontinuous blood exchange methods, resulting in high experimental costs and time, leading to high failure rates\cite{10, 20}. Recent studies have explored alternative methods for blood exchange in animals, such as transfusion of small animals through intermittent infusion by the use of microcirculation electronic pumps\cite{8, 22}. Although these research methods\cite{8, 22} have reduced experimental costs, simplified procedures, and increased experimental control compared to previous study\cite{14}, they\cite{8, 22} often require pre-implanted jugular veins, which can lead to serious surgical injuries. And those methods has limitations include intermittent plasma exchange\cite{8}, a small exchange volume per time\cite{23}.

An innovative technique for performing whole blood exchange in rats was developed during our review of research methods for blood exchange. This technique involves smaller surgical incisions, simpler implementation, and closer resemblance to clinical blood exchange procedures, with a blood exchange volume exceeding the rat's systemic circulation. Isoflurane anesthesia is used to insert an arterial catheter (as the blood outflow channel) into the femoral artery and a 24G catheter needle (as the healthy whole blood inflow channel) into the tail vein of the rat. Continuous monitoring of vital signs during anesthesia and ensures that the dual-directional syringe pumps control the inflow and outflow rates of fluid, resulting in successful whole blood exchange in rats. This method provides an experimental approach for further exploring the possible mechanisms of blood exchange therapy, and which exhibited a low incidence of postoperative infections and mortality rates.

1.1 Materials and Methods

1.1.1 Animals
In this study, healthy adult male Sprague-Dawley (SD) rats weighing between 290 and 340 grams, were sourced from China Charles River Laboratories. Prior to use in the study, the rats were screened and certified to be free of specific pathogens. These rats were housed in the Experimental Animal Department of Central South University, where they were maintained in a controlled environment with a 12-hour light/12-hour dark cycle and provided with ad libitum access to food and water, both of which were sourced from the same department. The rats were accommodated in pathogen-free cages, specifically designed for animal experiments, with each cage housing three rats. After a one-week acclimation period, during which their health was closely monitored, the rats were randomly assigned to their respective experimental groups.

This study was approved by the Central South University Experimental Animal Welfare Ethics Review Application and the Central South University Science Research Project Experimental Animal Welfare Ethics Approval (ethics approval number: CSU-2023-0065) and was conducted in compliance with all relevant regulations governing the ethical use of laboratory animals for research purposes. All experimenters obtained experimental permits through an ethical qualification examination. A routine ethical review was conducted prior to the commencement of the formal experiment to ensure that all procedures adhered to the highest standards of ethical conduct. And the study adheres to ARRIVE Essential 10 of the ARRIVE guidelines 2.0 including compliance in reporting in vivo experiments, animal details, sample size, procedures, randomization, blinding, and statistics, underscores the study's quality, transparency, and scientific applicability.

1.1.2 Reagents

Isoflurane (RWD Life Science Co., Ltd Shenzhen China), povidone-iodine (Mingde Co., Ltd Dezhou China), heparin [24] (MedChemExpress New Jersey USA) and 0.9% sodium chloride (CR Double-crane Co., Ltd Pingdingshan China) injection were used in this study as anesthetic, antiseptic, anticoagulant, and isotonic solution, respectively (Figure 1A).

Figure 1. Materials presented in the figure are the required materials for this experiment. (A) Reagents; (B-C) Equipment; (D) Surgical tools; (E) Tubing, syringes, needles and related equipment; (F) Pump external hardware and tubing.

Isoflurane was administered through a precision vaporizer to achieve proper anesthesia. Povidone-iodine was used for skin preparation before surgery. Heparin was used to prevent blood clotting during experimental procedures. 0.9% sodium chloride injection was used for fluid replacement and electrolyte balance maintenance during the experiments (Figure 1A). All solutions were obtained from reliable medical suppliers and prepared according to standard protocols.
1.1.3 Equipment

Enhanced small animal anesthesia machine (R540IE Enhanced Small Animal Anesthesia Device-Easy Fill, 0-4L RWD Life Science Co., Ltd Shenzhen China)

Separable infusion pump (DSC-B01/W150-B01 Baoding Acmer Precision Pump Co., Ltd Baoding China) (Figure1B)

Electronic weighing scale (Wuxinhengqi Co., LTD Jinhua China) (Figure1C)

Professional pet trimmer (Zhuochuang Co., Ltd Wenzhou China) (Figure1C)

Electronic timer (Baijie Co., LTD Huzhou China) (Figure1C)

1.1.4 Surgical tools

Shadowless surgical lamp (Medilan Medical Equipment Co., LTD Nanjing China) (Figure1D)

Fixation plate for small animal dissection table (Zhongkehuida Co., LTD Beijing China) (Figure1D)

Hemostatic forceps (ShangHai Medical instrument Co., LTD Shanghai China) (Figure1D)

Surgical scissors (ShangHai Medical instrument Co., LTD Shanghai China) (Figure1D)

Ophthalmic forceps (ShangHai Medical instrument Co., LTD Shanghai China) (Figure1D)

Ophthalmic scissors (ShangHai Medical instrument Co., LTD Shanghai China) (Figure1D)

Animal dissection auxiliary retractor (Dasijiaer Co., LTD Huaibei China) (Figure1D)

Micro Vannas scissors (Dasijiaer Co., LTD Huaibei China) (Figure1D)

Vascular clamp (Dasijiaer Co., LTD Huaibei China) (Figure1D)

Handle for animal surgical blade (Dasijiaer Co., LTD Huaibei China) (Figure1D)

Sterile plastic handle surgical blade 36#/S4 (Fu Yang Medical Suture Needle factory, Hangzhou China) (Figure1D)

1.1.5 Tubing, syringes, needles and related equipment

Disposable sterile syringe 5 mL (Guangzhou Jet Bio-Filtration Co., Ltd Guangzhou China) (Figure1E)

Disposable centrifuge tube 15 mL (Guangzhou Jet Bio-Filtration Co., Ltd Guangzhou China) (Figure1E)

Disposable centrifuge tube 50 mL (Guangzhou Jet Bio-Filtration Co., Ltd Guangzhou China) (Figure1E)
Single-use human venous blood collection container (Hunan sanli Co., Ltd Liuyang China) (Figure1E)

Absorbable surgical suture 5-0 (Jinhuan Medical Co., LTD Shanghai China) (Figure1E)

Single-use three-way connector (Jiangsu Huaxing Medical Devices Industry Co., Ltd. Yangzhou China) (Figure1E)

Sterile Acrodisc® leukocyte filter needle 5 MM (PALL Corporation New York USA) (Figure1E)

Medical breathable tape (Figure1E)

Medical gauze (Figure1E)

Medical cotton swab (Figure1E)

Sterile surgical gloves (Figure1E)

1.1.6 Pump external hardware and tubing

Disposable indwelling needle 24G for arterial and venous access in rodents (Jiangxi Huali Medical Instrument Co., Ltd. Ganzhou China) (Figure1F)

Disposable catheter 26T for arterial and venous access in rats (Dasijiaer Co., LTD Huaibei China) (Figure1F)

Cautions! Please note that the specific model names in materials may vary depending on the manufacturer and region. These medical Materials have been essential in ensuring the successful implementation of our study.

2.2 Main steps of the study

2.2.1 Reagent preparation

Prepare the required reagents for the experiment prior to commencement: prepare heparin at a concentration of 30 IU/mL using physiological saline and set it aside; prepare iodine solution and physiological saline (Figure1A).

Caution! All reagent preparation procedures should be conducted in a sterile laminar flow hood to prevent contamination.

2.2.2 Catheter and Pump preparation

Using the prepared 30 IU/mL heparin pre-soak the disposable rat arterial and venous catheters, connecting tubes, three-way stopcocks, and sterile medication dispensers, and arrange them in a sterile surgical field (Figure1E-F). Record the volume of heparin used for pre-soaking and ensure that the air bubbles are removed from the catheters, as their presence may cause harm to the experimental animals.

Caution! All catheters should be prepared on a sterile work surface.
2.2.3 Transfusion blood preparation

Preparation of the infusion fluid required for the study before commencing the formal experiment. The blood of healthy rats was utilized as the source of infusion whole blood, obtained via cardiac puncture under general anesthesia in this experiment. Anticoagulation was achieved by heparinization with heparin. The required volume of the infusion fluid is calculated using the commonly used approximate formula for rat blood volume, where total blood volume \( [25] (\text{ml}) \approx \text{body weight} (\text{g}) \times 0.06 \). It is noteworthy that a preliminary cross-matching process utilizing a fully matched allogeneic donor is imperative to avoid potential hemolytic reactions during the experiment and ensure its success.

**Caution!** ensure a successful cross-matching process with age-matched donors.

2.2.4 Anesthesia, medication, and animal preparation

The experimental rats were anesthetized with an anesthesia machine (Supplementary Video), then expeditiously positioned in a supine stance on a surgical table, and the depth of anesthesia was assessed. The groin region was disinfected with iodine and shielded with sterile drapes. Following an incision in the groin area, the femoral artery, along with the femoral vein and nerve, was exposed. The femoral artery was dissected bluntly, and two absorbable surgical sutures were placed on the femoral artery without knotting. The distal end was ligated with a surgical suture, a clamping vascular clip attached to the proximal end. A V-shaped incision was carefully created using microscissors. Subsequently, a disposable arterial catheter for rats was inserted through the incision and secured in place. The femoral artery catheterization procedure was completed by covering the incision site with sterile gauze soaked in physiological saline solution. The other end of the catheter was connected to a sterile infusion set, which was mounted on a separate syringe pump. (Figure1D-F)

The tail end of the rats was disinfected with iodine, and the area 1/3 from the end was selected for cannulation and fixation of the tail vein using a disposable 24G arterial and venous indwelling needle. The other end of the indwelling needle was connected to a three-way connector or infusion set (depending on the experimental purpose) containing whole blood/blood components, which was fixed on another syringe pump. (Figure1E-F)

Upon successful placement, the catheter is connected to the appropriate equipment and the exchange device is activated. The device connection for blood exchange was accomplished and the flow rate for blood collection/infusion was established (Figure 2).

**Figure 2.** The overall view of the whole blood exchange procedure in rats.
Caution! The catheter is meticulously inserted into either the femoral artery or tail vein of the rat with great technical dexterity and precision to minimize any potential discomfort or harm to the animal.

Throughout the procedure, close attention is paid to the time, blood flow rate, velocity, and volume of the exchange, all of which are meticulously monitored and recorded to ensure precise data collection and analysis. Once the circulatory volume blood exchange is achieved, the catheter is carefully removed, the femoral artery is ligated, and the incision site is thoroughly irrigated with saline following iodine disinfection. The incision is then sutured, and the anesthesia machine is turned off, allowing the rat to awaken gradually over a period of 5-20 minutes. The entire continuous blood exchange process lasts for approximately 70-100 minutes examination (Supplementary Video).

Caution! It is crucial to maintain equivalent collection and infusion rates (200-300 µL/min) to ensure a balanced fluid equilibrium in rats, and prevent the occurrence of severe pathological injuries, such as shock or heart failure, that may lead to experimental failure. Special attention must be paid to maintaining a steady balance of fluid inflow and outflow to prevent any potentially detrimental effects.

2.2.5 Postoperative Management

After completion of the continuous blood exchange, the surgical site was meticulously closed and sterilized. The rat was then allowed to recover consciousness and was subjected to routine prophylactic antibiotic therapy to prevent infection. To facilitate a rapid postoperative recovery, a warming pad was utilized. Upon completion of the experiment, the rats were subjected to euthanasia using a controlled carbon dioxide displacement rate of 50% volume per minute.

Caution! The experiments were performed with 400 IU·kg-1 heparin dosage in systemic anticoagulation: And under similar anesthesia conditions with isoflurane, to eliminate possible physiological variations. To ensure the well-being of the rats, continuous monitoring of vital signs was conducted throughout the experiment.

2.2.6 Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 9.4.1. Normal distribution of the data was assessed using the 2way ANOVA test. The authors responsible for data analysis were blinded to the experimental groups. The results are presented as mean ± standard deviation (SD). A P-value of less than 0.05 (*) was considered statistically significant.

3.1. Results

Animal Model and Experimental Design

To demonstrate the applicability of continuous whole blood exchange, we established the drug-induced hemolytic anemia (DIHA) model and applied it to our experimental scenario. The current study employed the minimum number of animals (each group n=3) necessary for statistical analysis, thereby minimizing the impact on experimental animal welfare. A total of 12 SD rats were utilized in this study. Among them, 3 rats served as the healthy blood donor group, while the remaining 9 rats underwent the following data monitoring. The 9 rats were randomly allocated into three groups: the healthy control group, the WBE treatment group, and the DIHA model group. The DIHA model was induced by administering intraperitoneal injections of Hydrazine hydrochloride to all groups, except for the
healthy control group. Blood samples were collected at consistent time points of Day -1, Day 0, Day 1, and Day 3 by experienced and designated experimenters. Subsequently, all samples were promptly transferred to Xiangya Hospital's transfusion department for analysis using the Mindray CL-600 blood routine analyzer, aiming to minimize data variability. Following the experimental timeline outlined in Figure 3.

**Figure 3.** Schematic demonstrating the experimental timeline.

### Establishment of the Drug-Induced Hemolytic Model and Baseline Parameter Assessment

On the day preceding the commencement of the whole blood exchange experiment (Day -1), retro-orbital venous blood samples were collected from all rats to assess baseline values of red blood cell (RBC) count, hemoglobin (HGB), white blood cell (WBC) count, neutrophil (NEUT), platelet (PLT). Clinical signs, including body weight (B.W.) and temperature were measured (B.T.). All the parameter data, presented as mean ± SD, has been documented in Table I for reference in this study. The results demonstrated no statistically significant differences in the levels of RBC, HGB, WBC, NEUT, PLT, B.W. and B.T. among the three groups (Figure 4A-H). Subsequently on Day 0, a drug-induced hemolytic model was established by administering intraperitoneal injections of Hydrazine hydrochloride to all groups except for the healthy control group. The data depicted in Figure 4 on Day 0 exhibited a marked reduction in RBC count (P=0.0189, P=0.0029) and Hb levels (P=0.0143, P=0.0139), accompanied by a substantial increase in WBC count (P<0.0001, P<0.0001) and NEUT count (P<0.0001, P<0.0001) compared to the healthy control group, indicating the successful establishment of the drug-induced hemolytic model.

### Effects of Whole Blood Exchange Therapy on Hematological Parameters

After the application of continuous whole blood exchange therapy, we conducted assessments on Day 1 and observed the WBE treatment group has a significant increase in red blood cells (RBC) (P=0.0343), and hemoglobin levels (P=0.0090), accompanied by a noteworthy decrease in white blood cells (WBC) (P=0.122) and NEUT count (P<0.0001) compared with DIHA model group. Moreover, a visual assessment of the rat's paws on Day 1 demonstrated a discernible difference in coloration, the paws of the WBE-treated group exhibited a more vibrant and ruddy appearance, whereas the DIHA group displayed a pallid, grayish discoloration (Figure 4H). On Day 3 post-treatment, the results of WBE treatment group still revealed a significant elevation in RBCs (P<0.0001) and hemoglobin levels (P<0.0122) compared to the DIHA model group. Meanwhile, WBC and NEUT count are no significant difference between groups on Day 3 post-treatment. Concomitantly, we conducted evaluations on the platelet count, body weight, and body temperature of the rats to assess the therapeutic efficacy of Whole Blood Exchange (WBE) intervention. Our results demonstrated no statistically significant variations
in platelet count, body temperature, or body weight between the experimental groups (Figure 4E-G). These findings collectively demonstrate the beneficial impact of whole blood exchange therapy in ameliorating anemia in DIHA rats. Additionally, it is noteworthy that the implementation of Whole Blood Exchange therapy exhibited no evidence of infection or discernible effects on coagulation function, body weight, or body temperature in the rat model.

The rats treated with whole blood exchange exhibited immediate signs of alertness and liveliness upon awakening from anesthesia. They demonstrated independent feeding ability, and the wounds resulting from the treatment exhibited mild clotting and exudation on the day, with evident improvement in wound healing over time.

Figure 4. Efficacy of Continuous Whole Blood Exchange Therapy in DIHA Rats. Assessment of RBC(A), HGB (B), WBC(C), NEUT(D), PLT(E), Body temperature(F) and Body weight before and after treatment (each group n=3). Photographs of the left hind paws of rats(H). Statistical analysis (\(P>0.05\), not significant, ns), asterisks denote significant differences, with * indicating \(P<0.05\), ** indicating \(P<0.01\) and **** indicating \(P<0.0001\).

4.1. Discussion

Animal research methods for blood exchange in currently indicate that the extensive use of jugular vein catheterization\[^9, 22, 29\] which is a highly invasive procedures may lead to significant injuries and generated enormous uncertainties during the experimental process (e.g., poor healing of the large wounds, difficulty in removing the catheter). In this study, we demonstrated the continuous...
arteriovenous blood exchange method by using the femoral artery-tail vein exchange circulation to achieve an exchange model which is more efficient.

Our study using male rats in this experiment was aimed to reduce the potential physiological data variability caused by gender differences. A successful catheterization of the leg artery or tail vein could be critical for this method. Femoral artery catheterization can be referred to the classic experimental operation video\(^{[30]}\). The femoral artery catheterization can result in relatively small wounds, while also facilitating postoperative animal care compared to the jugular vein catheterization. A catheter is left in the tail vein, which is shallower and easier to puncture could be easy to implement tail vein catheterization. Experimental animals can recover quicker from this operation without an additional skin incision, which incision can cause unnecessary injuries to experimental animals.

The continuous blood exchange process must be conducted with catheters free of air bubbles to prevent experimental animals from dying from air embolisms. The balance speed of fluid inflow and outflow is vital for success, we recommend that all fluid flow rates should be between 200-300uL/min for this experiment as the excessive speed may cause red blood cell damage in the blood, while slow speed may cause catheter blockage, both of which can lead to experimental failure.

There are some critical aspects of this study design that must be emphasized: Isoflurane induction concentrations must keeping the concentration at 3-4% and maintaining it at 1.5-2%, with a flow rate of 0.2 liters per minute, is recommended to ensure that the experiment proceeds smoothly\(^{[31-33]}\). Please ensure the stability of the physiological status especially the blood pressure and blood volume of the experimental animals during anesthesia, which will directly affect the difficulty of vascular cannulation. An animal temperature maintenance instrument should be used to ensure the body temperature of the rats. To prevent experimental failure caused by vascular leakage after surgery, please ensure that the vascular ligation is successful as well.

Nevertheless, some limitations remain in this study. Inserting the catheter into the femoral artery of rats was more difficult compared to the jugular vein and required a high level of proficiency from the experimental technician. As mice have limited blood circulation and smaller arterial catheter diameters, this study method is more suitable for larger animals.

This experimental method through femoral artery-tail vein catheterization follows the 3Rs principle, promoting animal welfare provides a more clinically relevant and ethically humane method for blood exchange in animal experiments. Catheterization of the femoral artery can result in a wound that can be minimized and ligated, allowing for the separation of the experimental animals from the catheter. Through tail vein catheterization which reduces the need for surgical wounds, minimizing the trauma and pain experienced by the animals during surgery. Our blood exchange procedures not only have the potential to reduce the invasiveness and trauma, but also provides a new research method for animal experiments and ultimately contributes to exploration in biomedical research.

The establishment of a continuous blood exchange model in rats via femoral artery-tail vein cannulation is a significant advancement in animal experimentation research. This refined method offers a more clinically relevant approach and simplifies the experimental procedure, reducing the burden on the animals. Currently, this protocol serves as a valuable tool for exploring the mechanisms of various animal disease models, and please anticipate exciting results in our forthcoming research endeavors.

**Data availability statement**
The data supporting the findings of this study are openly available in the research data file and Table 340. And the study adheres to ARRIVE Essential 10 of the ARRIVE guidelines 2.0 including compliance in reporting in vivo experiments, animal details, sample size, procedures, randomization, blinding, and statistics, underscores the study's quality, transparency, and scientific applicability.

Conflict of Interest

All authors read and approved the final manuscript and declare that there are no potential conflicts of interest regarding the research, their identities as authors, and/or publication of this manuscript.

Author Contributions

Ning Li designed the study and supervised the project. Siya Pei and Yanjie Wang performed the animal experiment and wrote the manuscript. Danyang Yan and Xiangjie Fu analyzed the data. Zhimin Zhang, Cheng Mei and Wenyu Yin operated blood cross-matching. Yuanyuan zhu, tani Lin and Yiran Zhou tested the blood routine of the samples.

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References


Table 1. Variations in physiological parameters over time.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Timepoint</th>
<th>Control Mean±SD</th>
<th>DIHA model Mean±SD</th>
<th>WBE Mean±SD</th>
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<tbody>
<tr>
<td>RBC</td>
<td>Day-1</td>
<td>8.1±0.10</td>
<td>7.5±0.39</td>
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<td></td>
<td>Day 0</td>
<td>7.7±0.27</td>
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<td></td>
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<td>6.9±0.21</td>
<td>3.1±0.13</td>
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<td>6.5±0.17</td>
<td>1.9±0.17</td>
<td>5.1±0.09</td>
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<tr>
<td>WBC</td>
<td>Day-1</td>
<td>12.3±2.16</td>
<td>10.8±2.88</td>
<td>9.4±1.07</td>
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<td>Day 0</td>
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<td>12.1±3.47</td>
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<td>14.4±2.36</td>
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<td>HGB</td>
<td>Day-1</td>
<td>151.0±2.94</td>
<td>149.3±5.25</td>
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<td>102.3±7.41</td>
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<td>76.7±2.87</td>
<td>117.0±4.90</td>
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<td></td>
<td>Day 3</td>
<td>147.0±2.16</td>
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<td>105.3±4.03</td>
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<td>NEUT</td>
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<td>0.7±0.16</td>
<td>0.7±0.23</td>
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<td></td>
<td>Day 0</td>
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<td>25.5±3.80</td>
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<td>PLT</td>
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<td>Day 1</td>
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<td>576.0±116.87</td>
<td>543.0±149.06</td>
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<tr>
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<td>Day 3</td>
<td>839.7±199.39</td>
<td>586.7±132.16</td>
<td>661.3±266.18</td>
</tr>
<tr>
<td>B.W.</td>
<td>Day-1</td>
<td>362.5±11.67</td>
<td>361.5±6.92</td>
<td>359.0±2.19</td>
</tr>
<tr>
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<td>Day 0</td>
<td>358.1±12.27</td>
<td>337.1±7.15</td>
<td>346.3±4.34</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>369.3±13.47</td>
<td>320.6±7.60</td>
<td>343.3±2.45</td>
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<tr>
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<td>Day 3</td>
<td>386.2±16.17</td>
<td>334.9±7.14</td>
<td>354.5±5.39</td>
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<tr>
<td>B.T.</td>
<td>Day-1</td>
<td>36.8±0.25</td>
<td>37.2±0.05</td>
<td>37.3±0.29</td>
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<td>37.1±0.12</td>
<td>37.1±0.36</td>
<td>37.0±0.09</td>
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<td>37.0±0.28</td>
<td>37.0±0.36</td>
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<tr>
<td></td>
<td>Day 3</td>
<td>36.9±0.33</td>
<td>36.4±0.14</td>
<td>36.6±0.05</td>
</tr>
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</table>
Table 1. Variations in physiological parameters over time. Mean ± SD of RBC, WBC, HGB, NEUT, PLT, Body weight (B.W.) and Body temperature (B.T.) before and after treatment (each group n=3).
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Video.mp4