Role of Innate Lymphoid Cells on Laboratory Mice Routine Handling Process and their Effect on the Other Immunological Parameters

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KEYWORDS
Innate Immunity, Innate lymphoid cells, Laboratory Mice, Hematological, Biochemical, Mucin.

ABSTRACT
Introduction: Lab mice typically belong to the Mus musculus species. They are the prevailing choice for mammalian research and are utilized for studies in genetics, physiology, psychology, medicine, and various scientific fields.
Aim of the study: The current research aimed to examine how frequently handling laboratory mice impacts their innate immune response through innate lymphoid cells, mucus secretion, as well as various physiological and biochemical blood parameters, and its potential implications on research outcomes.
Study design: Sixty female mice were used in this study all animals were hosted under the same condition and treated with the same rotational animal handling process in different periods including animal control oral gavage and intraperitoneal, sub courteous, and intramuscular injection. Blood samples were gathered at four different time points to analyze various hematological and biochemical parameters, including (RBC, Hb, PCV, WBC, ALT, AST enzymes, and urea levels, etc.) while colon tissue were collected and the immunohistochemistry and PAS staining were preformed to evaluate the innate lymphoid cells and mucin secretion at three level through the experimental period.
The results: Based on the findings, there were no notable variations in the levels of Hb and RBC. However, there was a noticeable increase in the WBC count for all animals after 4 minutes of handling, and the levels began to normalize after 10 and 30 minutes. As for the blood's biochemical parameters, including ALT, AST, creatinine, and urea, no details were provided. The results showed that there were no noteworthy alterations in these parameters throughout the entire experimental period.
Conclusion: The handling of the animals is one of the most important factors that can influence the experimental results in lab mice. The WBCs and samples should be collected at least 30 minutes after the handling procedure.

1. Introduction

Mice are commonly used in medical research and student projects due to their ease of breeding and handling, as well as their suitability for organ isolation, blood collection, and inducing specific diseases or syndromes (1). Despite the benefits mentioned earlier, the researcher encountered difficulties when working with mice in their experiments, particularly when it came to the timing and method of blood collection. Consequently, a number of universities and organizations have put a protocols for handling laboratory animals, all of which emphasize a humane approach and impose limitations on collection methods and quantities. (4)

By extensively mapping the genome of mice and gaining a detailed understanding of their immunological properties (5). researchers have standardized experimental models, enhanced reproducibility and enabling global comparison of results, ultimately decreasing the necessity for experiment repetition. In the fields of veterinary and human medicine, progress in transgenic studies and genetic focusing have unveiled fresh opportunities for addressing disease mechanisms and exploring alternative treatment avenues (6,7).

Several factors, such as lineage, genotype, and gender, along with age, diet, environment, and collection location, Effects on mice's hematological and biochemical parameters. In order to evaluate the impact of diseases on various organs, understanding physiological parameters and correctly interpreting serum hematological and biochemical levels is essential (8,9,10). These measurements can indicate an animal's health status, nutritional levels, iron deficiencies in hemoglobin, presence of infections, and help in tracking treatment progress and prognosis (11).

The study’s goal is to examine how various animal handling methods impact Innate lymphoid cell three and Mucous secretion in mice, as well as certain physiological and biochemical parameters, in order to determine the optimal sampling method and time without affecting other experimental outcomes.

2. Materials and Methods

The experiment was carried out at the Faculty of Veterinary Medicine College/University of Basrah. A total of sixty female mice at an age of 8-10 weeks and weighing 20- 25 gm were used. The mice were housed at a controlled temperature of 22 °C and humidity of 50-60 %. There was a regular 12 hour cycle of dark and light (lights on at 7 a.m., lights off at 7 p.m.). Standard dietary rations and water were provided to all experimental animals. A total of four groups were formed (n=5 per group).

All experimental Swiss mice underwent a common daily animal handling process that used process included oral gavage, Enema, enter potential, and subcutaneous injection. the blood samples were collected at different periods after the animal handling process by using a sterilized method from the tail vein(16). the blood samples were collected at four different periods during the experiment time including first at time 0 right before the start animal handling process, and after that the blood was collected after 1 min, 10 min, and 30 min respectively. Using a 1ml disposable syringe, blood samples were collected through tail vein puncture under a sterilized process(9). An EDTA-treated tube was used for hematological parameters, and sterile labeled tubes (Clot Activator with Gel) were used for serum. preparation and biochemical analysis. An A complete blood count CBC performed by automated veterinary hematology counter (the pocH-100iV DiffLAM Sysmex® - Roche) was conducted to ascertain the all blood values determined before each analysis using control blood and an external quality evaluation program was implemented (17).

The blood serum was subjected to clinical biochemistry parameters using an automated spectrophotometer, the VITROS® 350 Chemistry System. The manufacturer's guidelines were followed when using the biochemical kits and calibration controls. All biochemical kits were purchased from Lab Test Diagnosis (18).

Periodic acid–Schiff stain (PAS) was used to stain serial sections of tissue fixed in paraffin resin. Goblet cells were stained, and alterations in mucose and mucin secretion were seen. The % involvement of each of the three following histologic traits was multiplied by the percent area of involvement to determine the histology score (19).

Immunohistochemically staining Some sections of mouse colon tissue were incubated with antibodies against inducible ILC3. The avidin-biotin indirect immunoperoxidase method was used for immunohistochemistry of paraffin-embedded sections (20). The Vectastain Elite ABC kit was used according to the manufacturer’s instructions (Vector Laboratories, Burlingame, CA).

The statistical analyses were performed using GraphPad Prism® 8 software (San Diego, CA). The trials were carried out in triplicate. One-way ANOVA was utilized for statistical comparisons unless otherwise specified, and the Mann-Whitney test was employed to examine variations among non-parametric groups. $P<0.05$ was used to determine the significance of the differences.

3. Results:

The complete blood count of mice blood revealed no significant changes in normal erythrocyte indices levels the other parameters varied according to the time of blood collection after Routine Lab animal handling (Fig1). PLT counts found in mice were similar at all blood collection periods (fig 2). The automated leukocyte count showed that mice have a greater increase in the number of immune cells in the first minute after the routine Lab animal procedure. However, the differential leukometry data showed that significant increases in the numbers of LYN, MON, and NEU/SEG were found in the first minute respectively, However, all analyses revealed that the level of leukocyte was returned to normal after 10 min, and 30 min of routine Lab animal procedure (Fig 3). The biochemical for blood serum analyses demonstrated no significant changes in AST, ALT, UR, AP, GLB, CHL, ALB, and TG at any of experiment period. (Fig 4). the immunohistochemistry examination for colon tissue revealed there a significant increase in the number of innate lymphoid cells type three ILC3 in the colon lamina propria of mice that underwent blood collection after 1 minute however the ILC3 seemed to return to normal in the mice that underwent to blood collection after 10 and 30 minute of experiment period (Fig 5). the examination of mucous secretion from colon goblet cells revealed there a significant increase in the size and number of goblet cells due to the rise of mucin secretion in the colon mucosal layer of mice that underwent blood collection after 1 minute however the size and number of goblet cells seemed to return to normal in the mice that underwent to blood collection after 10 and 30 minute of experiment period (Fig 6).

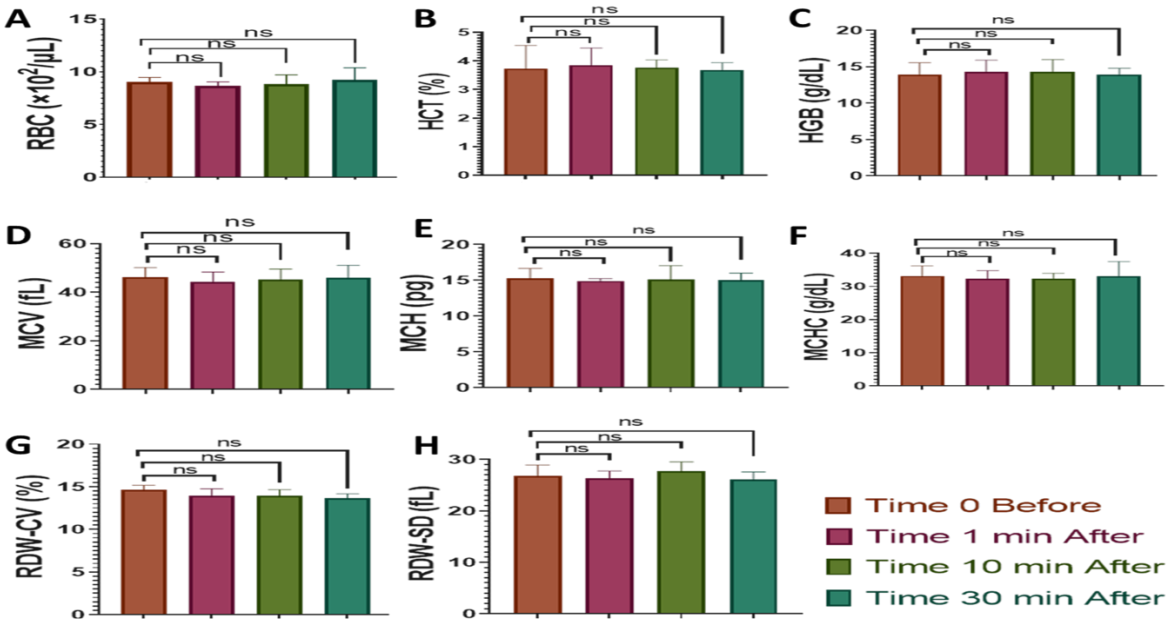

Fig (1): Routine Lab animal handling changes normal erythrocyte indices levels:
A) RBC, red blood cell ; **B)** HCT, hematocrit; **C)** HGB, hemoglobin concentration; **D)** MCV, mean corpuscular volume; **E)** MCH, mean corpuscular hemoglobin; **F)** MCHC, mean corpuscular hemoglobin concentration; **G)** RDW-CV, red cell distribution width - coefficient of variation; **H)** RDW-SD, red blood cells dimension width - standard deviation. **CBCs** were performed in an automated veterinary hematology counter pocH-100iV Diff™ (Sysmex® - Roche). Data show means ± standard Error Mean (SEM), calculated with GraphPad Prism version 8.01. Significance (P-value: * < 0.05, ** < 0.01, *** < 0.005, **** < 0.001) was determined by using 1-way ANOVA and post hoc Tukey's test. In all data presented in the figure, 5 mice were used in each group. The data presented are representative of at least 3 independent experiments.

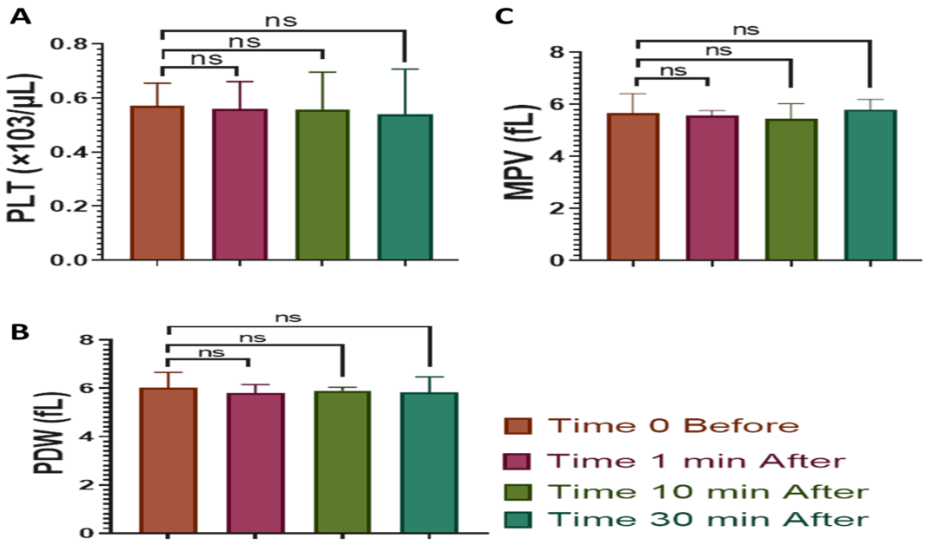

Fig (2): Routine Lab animal handling changes normal platelets indices levels:
A) PLT, number of platelets; **B)**PDW, platelet dimensions width.; **C)** MPV mean platelet volume; . **CBCs** were performed in an automated veterinary hematology counter pocH-100iV Diff™ (Sysmex® - Roche). Data show means ± standard Error Mean (SEM), calculated with GraphPad Prism version 8.01. Significance (P-value: * < 0.05, ** < 0.01, *** < 0.005, **** < 0.001) was determined by using 1-way ANOVA and post hoc Tukey's test. In all data presented in the figure, 5 mice were used in each group. The data presented are representative of at least 3 independent experiments.

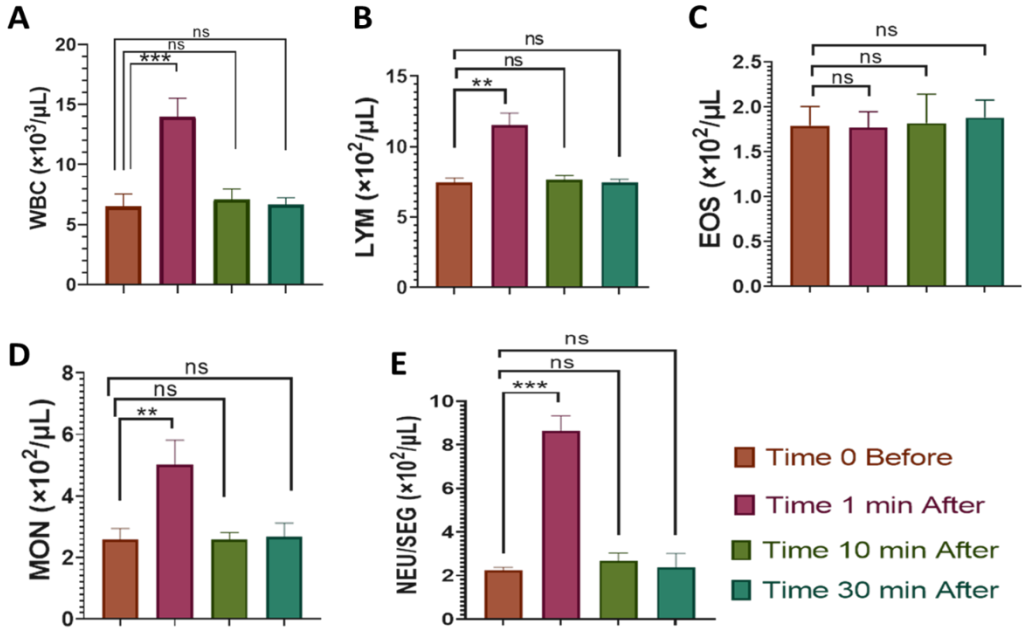

Fig (3): Routine Lab animal handling changes normal leukocyte indices levels:
A) WBC, number of white blood cells ; **B)** LYM, lymphocytes; **C)** EOS, eosinophils; **D)** MON, monocytes; **E)** NEU/SEG, neutrophils/Segmented. **CBCs** were performed in an automated veterinary hematology counter pocH-100iV Diff™ (Sysmex® - Roche). Data show means ± standard Error Mean (SEM), calculated with GraphPad Prism version 8.01. Significance (P-value: * < 0.05, ** < 0.01, *** < 0.005, **** < 0.001) was determined by using 1-way ANOVA and post hoc Tukey's test. In all data presented in the figure, 5 mice were used in each group. The data presented are representative of at least 3 independent experiments.

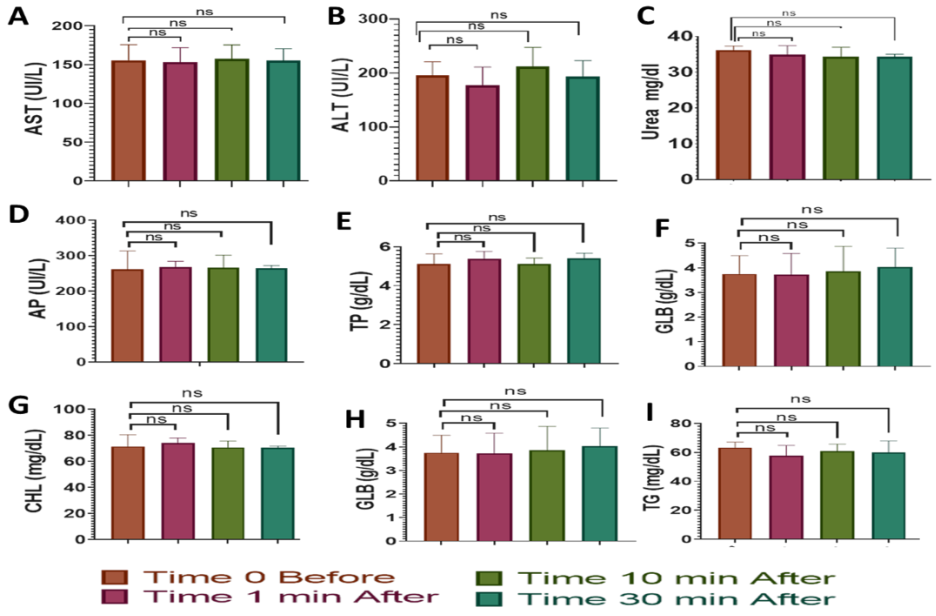

Fig (4): Routine Lab animal handling changes serum biochemical indices levels:
A) AST, aspartate transaminase; **B)** ALT, alanine transaminase; **C)** UR, urea; **D)** AP, alkaline phosphatase; **E)** TP, total protein; **F)** GLB, globulin; **G)** CHL, cholesterol; **H)** ALB, albumin; **I)** TG, triglycerides...Sera were processed by(automated spectrophotometer). Data show means ± standard Error Mean (SEM), calculated with GraphPad Prism version 8.01. Significance (P-value: * < 0.05, ** < 0.01, *** < 0.005, **** < 0.001) was determined by using 1-way ANOVA and post hoc Tukey's test. In all data presented in the figure, 5 mice were used in each group. The data presented are representative of at least 3 independent experiments.

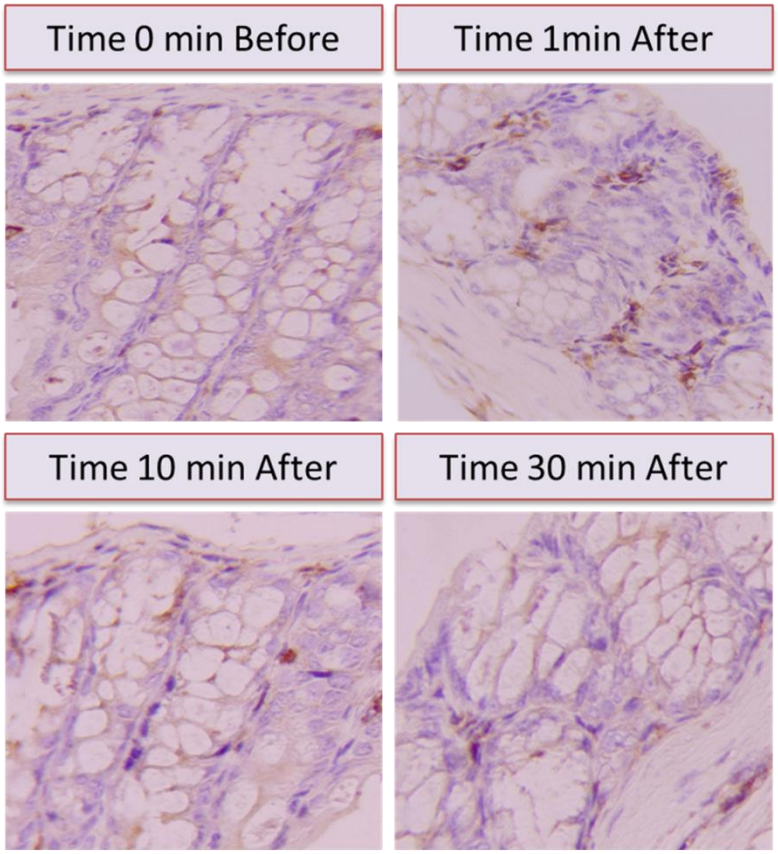

Fig (5): Routine Lab animal handling changes the colon resident innate lymphoid cells levels: (ICH) Immunohistochemistry were applied on colon tissue to target level of **(ILC)** innate lymphoid cells in the colon. :

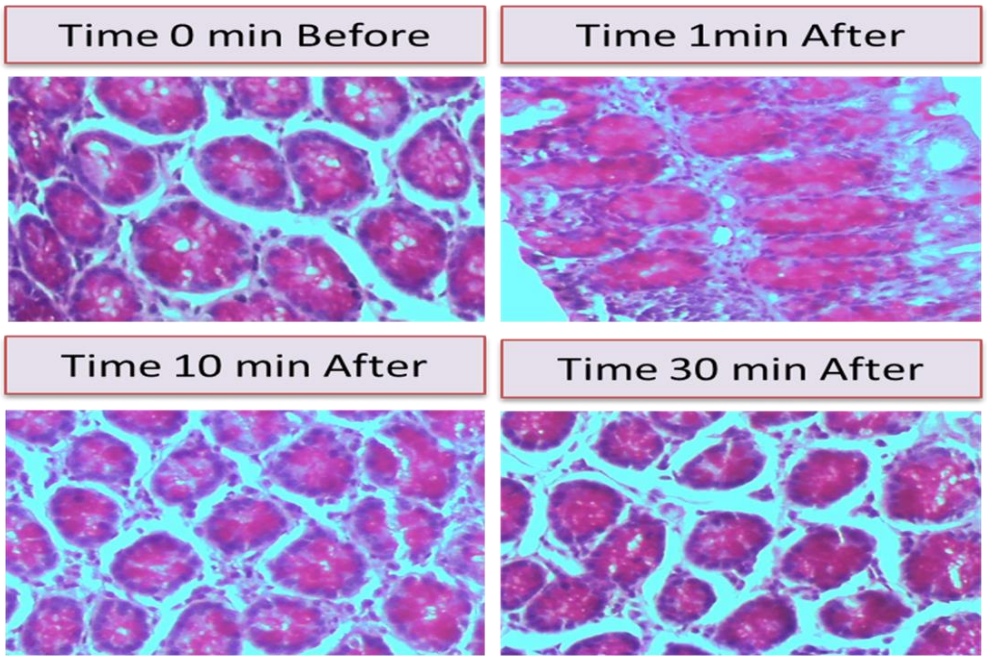

Fig (6): Routine Lab animal handling changes the colon resident Goblet cells and mucous levels: Periodic acid-Schiff stain **(PAS)** were applied on colon tissue to target level of mucin in the colon Goblet cells :

4. Discussion and Conclusion

The WBC count and the innate immune response, indicated by mucus secretion and an increase in ILC3, significantly increased ($P<0.05$) within the first minute after the experiment in all animal groups compared to before the experiment began. Hb, RBC, and PCV % values showed consistency across all groups. The reason for increased total WBCs due to the activation of internal immune response is a significant reaction caused by handling animals, which could harm the host's defense system by leading to excessive production of inflammatory substances (12). howover, the reaction only lasts temporarily and typically returns to normal within 10 to 30 minutes of the handling process. The reason for this shift is the increase in elevation and the decrease in stress, which also affects any other changes associated with handling (13). AST is a enzyme located inside cells, found in both the cytoplasm and mitochondria (1,14). ALT, AST, urea, and creatinine concentrations in mice are present in different organs and tissues such as the liver, kidneys, heart, brain, skeletal muscle, and erythrocytes. Throughout all experimental periods, the enzymes take longer than anticipated to reach elevated levels (1,15). however, Extended periods of stress can have an impact on how well the liver and kidneys operate (12,13, 15).

In conclusion: Proper timing for sample collection is crucial in reducing handling stress on lab mice, particularly on white blood cells, as it can significantly impact experimental results. Waiting at least 30 minutes after handling is recommended for more physiologically relevant conditions.

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