

Acute and Chronic Toxicity of Thioacetamide and Alterations in Blood Cell Indices in Rats

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ABSTRACT

Background: Thioacetamide (TAA) has been used extensively in the development of suitable animal models of acute and chronic liver injury employing various doses, times and routes of its administration, particularly in drinking water due to its resemblance with human liver fibrosis and cirrhosis. The aim of this study was to investigate and compare hematological alteration during the acute and chronic liver inflammation. **Methods:** Acute Liver inflammation was induced in Wistar rats via intraperitoneal injection of thioacetamide and the animals were sacrificed 12 h after the TAA administration. Induction of chronic liver inflammation was performed by continuous administration of TAA in the drinking water (200 mg/L) during 18 weeks of experiment. After that all animals were sacrificed and Blood samples were collected for further analysis. **Results:** Single intra peritoneal injection of TAA (300 mg/kg B.W.) induced an acute condition with hematological changes including leukocytosis with marked neutrophilia ($P = 0.0429$), lymphopenia, thrombocytosis as well as increased hemoglobin concentration ($P < 0.05$) and decline of erythrocytic count ($P = 0.0009$). Eighteen weeks of uninterrupted supply of TAA (200 mg/L) in drinking water lead to chronic inflammation and the hematological alterations were leucopenia ($P = 0.0197$) accompanied with neutropenia and thrombocytopenia. Increase in RBCs ($P = 0.0073$) and Hb contents was also observed with a decline of red cell indices. **Conclusion:** Taken together these findings we can conclude that the animals respond differently under acute and chronic inflammatory condition with TAA administration. Leukocytosis with marked neutrophilia, thrombocytosis as well as increased hemoglobin concentration and decline of erythrocytic count were observed in acute while leucopenia accompanied with neutropenia and thrombocytopenia and increase in RBCs, Hb and Hct was also observed with a decline of other red cell indices during chronic phase.

Keywords: Haematology; Inflammation; Leucocytosis; Leucopenia; Neutrophilia; Neutropenia; Thrombocytosis and Thrombocytopenia

1. Introduction

Thioacetamide (TAA), a potent hepatotoxin, was first used to control the decay of citrus fruits especially oranges and then as a fungicide. Various industrial as well as pharmaceutical applications, TAA acts as sulfur donor. Later it was found that it serves as a potent chemical carcinogen [1-4].

TAA induced inflammation leads to cirrhotic conditions in the rat liver that resembles the human alcoholic liver fibrosis. It is a model hepatotoxicant and responsi-

ble of severe damage to the cells with significant toxic effects on biosynthesis of macromolecules including proteins and nucleic acids [5]. The injury to the liver cell population is due to increased production of TAA metabolites including sulfoxides and sulfene [6,7]. It has been reported that TAA can also injure different organ systems besides liver, including lungs, intestine, kidneys, spleen, thymus and pancreas [8-13].

TAA is bio-transformed or metabolized by flavin-containing monooxygenase (FMOs) systems [14] and cytochrome P450 (Cyt-p450) [4]. FMOs and Cyt-p450 reduce dioxygen to superoxide anion, which is catalyzed

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to form hydrogen peroxide [15]. Enzymes of Cyt-p450 are present in the liver microsomes, which converts TAA to a toxic reactive intermediate called thioacetamide S-oxide (TASO) & due to oxidation process it induces oxidative stress in the hepatic cells that finally leads to centrilobular necrosis and liver injury [1,16-19]. It also provokes a remarkable increase in the size of the nucleoli, as well as in the amount and activity of total mRNA of liver cells [20].

TAA has been extensively used for several years to establish a model of acute & chronic liver injury in rats, mice and other animals [21-23], utilizing its various doses (50 - 600 mg/Kg B.W.) and routes of administration [24-28] particularly in drinking water [29-32] and in the diet [17] because of the fact that the clinical conditions, biochemical and histological alterations in TAA-induced models have shown high similarity to human liver failure [33-41].

Acute toxic effect of TAA is dose dependent as single dose leads to injury specified to centrilobular region of the liver with subsequent regenerative response [33,41, 42]. Long-term thioacetamide administration is also an established technique for generating rat models of liver fibrosis and cirrhosis [15]. Prolonged oral intake of this chemical exhibited an external appearance of cirrhosis characterized by the presence of; numerous macro liver nodules, cell adenomas, cholangiomas and hepatocarcinoma, histologically similar to that caused due to viral hepatitis infection [20,43,44].

Little is known regarding changes in the blood cell indices during acute or chronic inflammation induced by TAA. Therefore, the aim of the current work was to compare hematological alterations of acute and chronic experimental inflammation using TAA as a model hepatotoxicant.

2. Materials and Methods

2.1. Animals

Healthy Wistar rats (about 300 g B.W.) were arranged from the Department of Zoology, GCS (Lahore-Pakistan), housed in wire-bottomed cages in an animal room under standard conditions with 12-h light/dark cycles and at an ambient temperature of $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$, with fresh water and food pellets available *ad libitum*. All the animals were acclimatized under standard laboratory condition for a period of 2 weeks before the commencement of the experiment.

2.2. Acute Inflammation

Acute Liver inflammation was induced in Wistar rats via intraperitoneal injection of thioacetamide (Sigma Aldrich chemie, Switzerland; 300 mg/kg of B.W.), however, the

control rats received injections of normal saline ($n = 3$). The animals were sacrificed 12 h after the TAA administration.

2.3. Chronic Inflammation

Induction of chronic liver inflammation was performed by continuous administration of TAA in the drinking water (200 mg/L) during 18 weeks of experiment whereas the control group received tap water.

All the animals were anesthetized with intra-peritoneal injection of ketamine-distilled water mixture (1:1), (50 mg/ml of ketamine) and scarified. Blood of the control and treated animals, was drawn through direct cardiac puncture and processed for the measurement of blood cell indices.

2.4. Assessment of Hematological Parameters

Complete blood counts were performed on the samples of control and treated animals, using an automated blood cell analyzer Sysmex XT-1800i (Japan). It utilizes the technology of fluorescent flow cytometry and hydrodynamic focusing. Fluorescent technology consistently differentiates normal white blood cells (WBCs), Red blood cells (RBCs) and platelets (plts) from abnormal populations, thereby decreasing the number of manual interventions.

2.5. Statistical Analysis

The data were analyzed using Prism Graph pad 5 software (San Diego, CA). Statistical significance was calculated by two-sided t-test to assess differences against the control group. Significance was accepted at $P < 0.05$, $n = 3$.

3. Results

3.1. Effect of Acute and Chronic Administration of TAA on Serum Level of Total and Differential Leukocyte Count

A rise of 45% in Total Leukocyte count (TLC) compared to the respective control was observed in acute group (**Figure 1(a)**) while a similar percentage decline was noted in chronic group ($P < 0.05$) (**Figure 1(b)**). Circulating level of subpopulation of WBCs was also analyzed. Neutrophils exhibited a marked statistical increase of 283% in acute group ($P = 0.0429$) and decrease of 47.97% in chronic group ($P = 0.0391$) (**Figures 1(c)** and **(d)**). The rest of the WBCs population in both of the studied groups showed no statistically significant change. Lymphocytes and monocytes showed a decrease of 20 and 94.25% compared to control during acute condition, while an increment of 34.60 and 9.52% was noted in the

parameter during chronic condition. A general increase was also seen in eosinophils in both the experimental groups (Figures 1(e) and (f)).

3.2. Assessment of Red Cell Indices and Platelets during Acute and Chronic Administration of TAA

Blood level of platelets reflects an increment of 44% and decline of 6% in acute group and II, respectively compared to their respective controls (Figures 2(a) and (b)).

Mean cell volume (MCV), hematocrite (Hct), Mean cell haemoglobin concentration (MCHC) and Mean cell haemoglobin (MCH) were studied. Except for Hct, all the other indices reflected an increasing trend in acute group while more or less a steady level with a minor decrease was observed in chronic group. Hct showed a statistically significant elevation in chronic group ($P = 0.0001$) while 27% decrease was noted between experimental and control samples in acute group ($P < 0.05$) (Figures 3(a) and (b)).

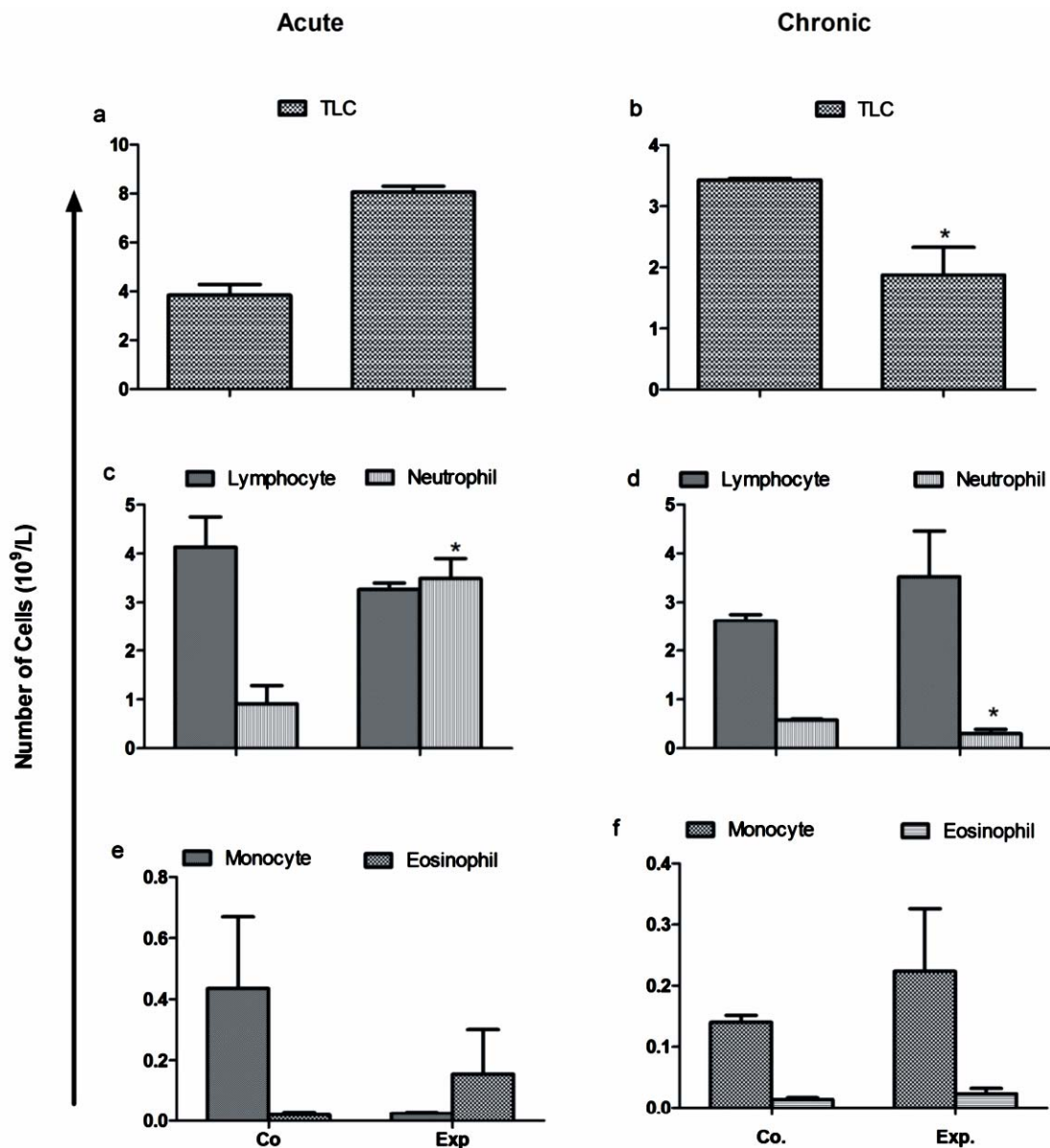


Figure 1. Total leukocyte count ($10^9/L$) in the treated animals as compared to control animals. A rise of 45% in TLC compared to the respective control was observed in acute group while a similar percentage decline was noted in chronic group ($P < 0.05$). Neutrophils exhibited a marked statistical increase of 283% in acute group ($P = 0.0429$) and decrease of 47.97% in chronic group ($P = 0.0391$). The rest of the WBCs population in both of the studied groups showed no statistically significant change.

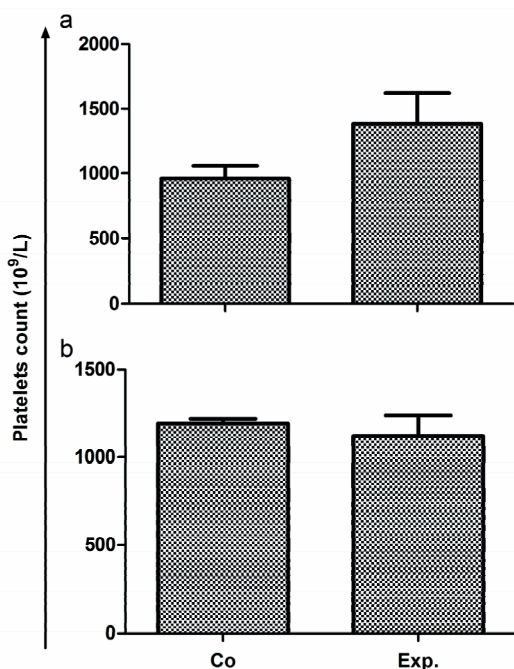


Figure 2. Platelets count (10⁹/L) in the treated animals against control animals. Blood level of platelets reflects an increment of 44% and decline of 6% in acute group and chronic group, respectively compared to their respective controls.

Haemoglobin (Hb) contents increased after TAA treatment in both groups. A statistical significant rise was observed in acute group ($P < 0.05$) whilst minor increase of 4.1% was also noted in chronic group (Figures 3(c) and (d)). Regarding Red blood cells (RBCs), a significant decline was shown in acute group ($P = 0.0009$) on the contrary to chronic group which showed a rise of 11% compared to the control ($P = 0.0073$) (Figures 3(e) and (f)).

4. Discussion

Thioacetamide (TAA) administration either intraperitoneally or in drinking water up to 6 months produced liver cirrhosis and hepatic neoplasms in various animal models with significant biochemical and histological alterations, similar to those observed in human cirrhosis and it has been extensively investigated and reported in the scientific literatures [21-25,27,28,32-34,38,39].

In the present study, the hematological alterations of acute and chronic experimental inflammation in adult wistar rats were monitored because of their diagnostic significance. Intraperitoneal (300 mg/kg) acute group after 12 h and (200 mg/L) in drinking water up to 18 weeks, chronic group of TAA were shown to have dose-dependent effects on blood profile of both of the animal groups.

The acute inflammation increased blood indices in-

cluding Hb, platelet levels, MCV, MCHC, MCH, TLC, neutrophils and decreased lymphocytes, monocytes, Hct and RBCs. On the contrary, during chronic phase, an increment was noted for RBC, Hct and Hb contents, remaining parameters have shown opposite results to that of acute inflammation.

Leukocytosis occurs in response to a wide variety of conditions, including viral, microbial infections, cancer, hemorrhage, and exposure to certain chemicals including steroids. The mechanism that causes leukocytosis includes: an increase, in number of leukocyte precursor cells in the bone marrow or their increased release from bone marrow storage pools, decreased margination of leukocytes into vessel walls and their decreased extravasation from the vessels into tissues [45]. Leukocytosis observed in case of acute group might be due to tissue necrosis or infarction resulted due to exposure of lethal dose of TAA. Decrease in RBCs count and Hb content leads to iron deficiency anemia which is characterized by a microcytic hypochromic blood picture [46,47].

The depression in RBCs count and rise in Hb contents and red cell indices including MCV, MCHC, MCH, recorded in the present work in case of acute group is clear suggestive to a megaloblastic picture of RBCs and could be attributed to disturbed hematopoiesis, destruction of erythrocytes, and reduction in the rate of their formation and/or their enhanced removal from circulation. According to Travlos *et al.* (1996), consistent erythrocyte damage is presumed to be related to direct oxidative injury to the red cells by the chemicals or to the pitting function of the spleen. It may be assumed that the free radicals resulting from TAA metabolism caused liver injury [48].

Leucopenia is a clinical manifestation with decrease in circulating white blood cell (WBC) and is usually characterized by low granulocyte number, particularly the blood neutrophils. This notion is evident from the results observed in chronic group with a significant negative change of 47.97% in the level of the neutrophils and 6% of thrombocytes. These results could be attributed to a rare case of hematologic side effects called neutropenia and thrombocytopenia often associated with antipyretic drug overdose like paracetamol [49,50]. Hematological stem cells destruction due to underlying malignancies, acute and chronic disorders of alcohol abuse also causes thrombocytopenia [51,52]. Hypersplenism contribute to the thrombocytopenia if cirrhosis of the liver is present as well. Leucopenia and increased RBC and Hb contents were observed with the administration of 50 mg/kg of ciprofloxacin and pefloxacin in rats inflamed with formaldehyde [53]. Similar results were supported by Moura *et al.* [54] with the administration of 500 mg/kg of the leaf extract of *Ageratum conyzoides* in chronic (formaldehyde-induced arthritis) models of inflamed rats. Radiations, endogenous or exogenous chemicals and microbial

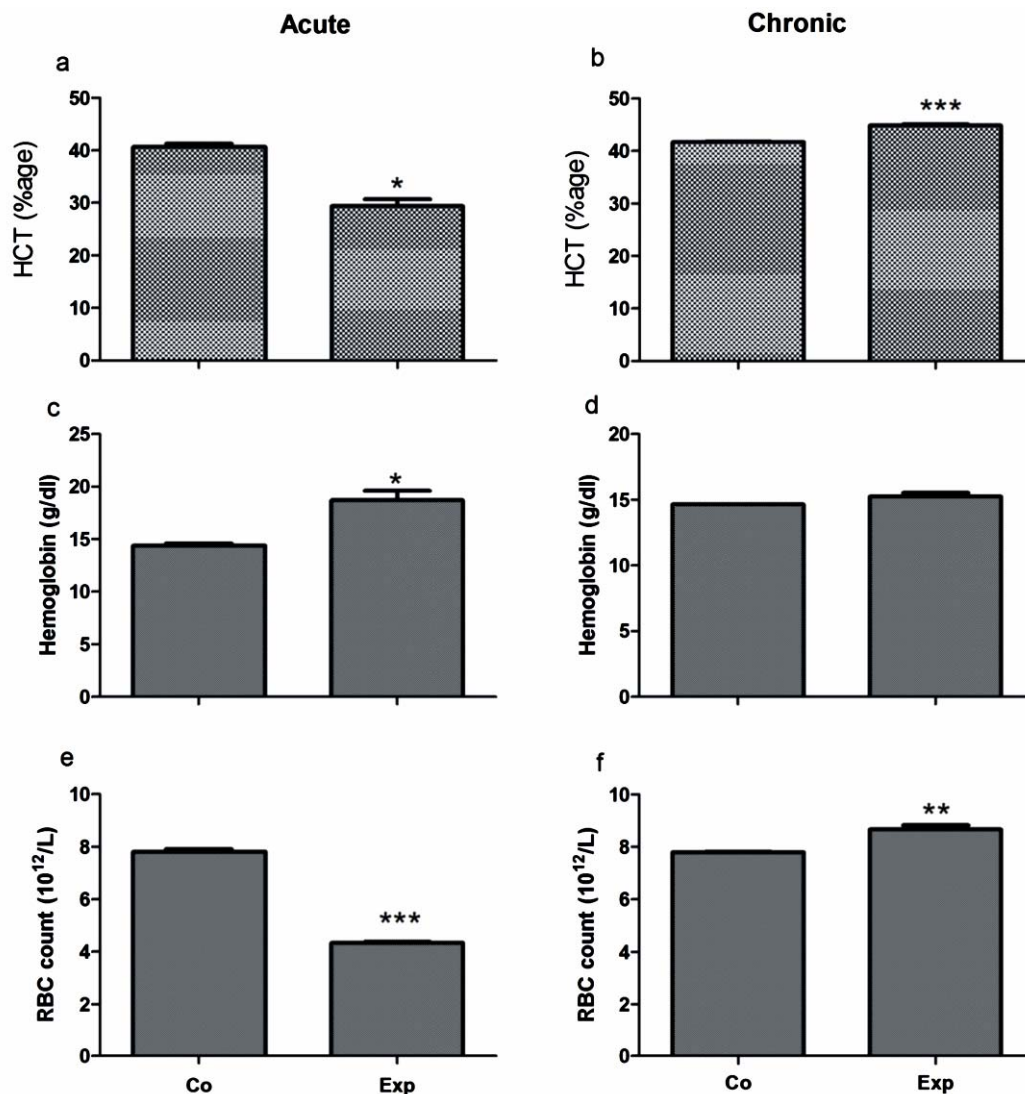


Figure 3. Estimation of hematological parameters in the treated animals against control animals. Hct showed a significant elevation in chronic group ($P = 0.0001$) while 27% decrease was noted in acute group ($P < 0.05$). A significant rise of Hb contents was observed in acute group ($P < 0.05$) whilst minor increase of 4.1% was noted in chronic group. Regarding RBCs, a significant decline was shown in acute group ($P = 0.0009$) on the contrary to chronic group which showed a rise of 11% compared to the control ($P = 0.0073$).

agents has induced chronic inflammation which is usually associated with cancer development [55,56]. 80 mg Earthworm paste/kg treated rat showed same trend of WBC, RBC and Hb contents [57]. Falling in line with these observations it was found that in the present study, 200 mg/L of TAA treated group showed reduction of 45% in WBCs (Leucopenia) and increase of 11 and 4.1% of RBC and Hb contents, respectively (polycythemia). A transient increase of RBC and Hb contents were registered together lymphopenia in rats intramuscularly injected with 5 ml/kg of *Nerium oleander* aqueous leaf extract (unpublished data). Chronic group in this study showed similar results and are in accordance with previous data.

Neutrophils and its derived cytokines play a crucial role in the development and manifestation of inflammation. The stimulation of neutrophils can lead to the production of oxygen-derived free radicals also called reactive oxygen species (ROS) that cause further cellular damage [58]. The formation of free radicals and cytotoxic oxygen metabolites probably impart a key role in various types of tissue degeneration and pathology such as aging, cancer and retinal degeneration [33,55,59]. In the present study, after intraperitoneal injection of TAA, significant elevation in the neutrophil count (neutrophilia) occurs with 283% rise in its level compared to control may be due to the free radicals resulting from TAA metabolism which caused liver injury and a proportion of

these free radicals librated into the blood may also affect the circulating cells and induced a significant change in their number. This significant neutrophilia might reflects its involvement in inflammation by forming various reactive oxygen species (ROS), inflammatory, metabolic and myloproliferative disorders, tissue necrosis, acute hemorrhagia, malignant tumors or due to rapid release of young cells from the bone marrow [60]. Similarly, in previous reports, significant neutrophilia, and decrease of circulating lymphocytes, remaining below control levels after 4 h was observed with intramuscular turpentine oil treatment of rats [61]. This notion was indicated by the acute group in this study and in accordance with previous data [62].

5. Conclusion

Taken together these findings we can conclude that the animals respond differently under acute and chronic inflammatory condition with TAA administration. Leukocytosis with marked neutrophilia, thrombocytosis as well as increased hemoglobin concentration and decline of erythrocytic count were observed in acute while leucopenia accompanied with neutropenia and thrombocytopenia and increase in RBCs, Hb and Hct was also observed with a decline of other red cell indices during chronic phase.

6. Author's Contributions

Muddasir Hassan Abbasi involved in the experimental work, data analysis and manuscript draft.

Tasleem Akhtar involved in the chronic treatment experimental work, data collection and manuscript draft.

Ihtaz A. Malik participated in the study design.

Sana Fatima participated in interpretation of results.

Babar Khawarand Ghulam Mustafa involved in acute treatment experimental work.

Khawaja Abdul Mujeeb and Javaid Iqbal involved in trouble shooting suggestions and discussion for acute experiments.

Shabir Hussain performed hematology.

Nadeem Sheikh provided the concept, participated in the design of study, supervised the manuscript draft and approved the final manuscript.

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List of Abbreviations:

B.W. Body weight
 FMOs Flavin-containing monooxygenase
 Hb Haemoglobin
 Hct Hematocrite
 MCH Mean cell hemoglobin
 MCHC Mean cell hemoglobin concentration
 MCV Mean cell volume

TAA Thioacetamide
 TLC Total leukocyte count
 TASO Thioacetamide-S-oxide
 Plts Platelets
 RBCs Red blood cells
 ROS Reactive oxygen species
 WBCs White blood cells