Effect of Oral Administration of Tungsten Trioxide (WO3) Particles on Hispathological Feature of liver and kidney in Rat

H S H Munawaroh*1, A B D Nandiyanto1, G G Gumilar1, A Widi1 and M Subangkit5

1Departemen Kimia, FPMIPA Universitas Pendidikan Indonesia
Jl. Dr. Setiabudhi No. 229 Bandung 40154, Jawa Barat-Indonesia

2Fakultas Kedokteran Hewan, Institut Pertanian Bogor
Jl. Agatis, Kampus IPB Darmaga, Bogor, Jawa Barat-Indonesia

*heli@upi.edu

Abstract. This study aims to investigate the toxicity and histopathology of tungsten trioxide (WO3) administration on rat’s liver and kidney. The LD50 of WO3 was determined and the sub acute toxicity was evaluated by orally administration of 5000 mg kg⁻¹ of WO3 to rat for 14 consecutive days. Parameter of blood cells, ALT, creatinine, and BUN were experimentally measured. The toxicological evaluation showed that WO3 is a non toxic compound with the LD50 higher that 5000 mg kg⁻¹. No biochemical change was observed for creatinine and Blood Urea Nitrogen parameter. In contrast, ALT parameter shows higher value in the experiment than that in the control group. Histopathological changes on rat’s liver and kidney were also studied. Small defects in rat’s liver and kidney were found, which may interfere the functional of related enzymes.

1. Introduction
Tungsten, originally known as wolfram, is one of the rarer metals which has some excellent properties among other elements, such as relative harmlessness, chemical and thermal stability [1], chemical and biological inertness [2], relative hardness, and second highest melting point of any element [3][4]. Tungsten trioxide (WO3) is one of the derivates of tungsten (wolfram), which is frequently used in many industrial sectors, such as pigments in ceramics and paints, and color-resistant mordant for textile and fireproofing fabrics, [5][6]. Tungsten transition compound is also used in many millitary applications, since it has the highest melting point of any elements.

The increased applications of tungsten compounds may result in the release of tungsten to the environment. Further, through food chain, they may accumulate in living organisms, causing serious problems. The presence of this compounds in the body can have various toxicological consequences,
including acute toxicity and pathological of any tissue. However, very limited data are available on occupational exposure of tungsten trioxide compounds the information about its potential toxicity to living organisms and its environmental impact is of great interest.

Based on our experience in the use of tungsten and its derivatives compounds,[7][8][9][10] this study aims to investigate effects of WO3 toxicity and histopathological on living organisms. Since the derivatives of sodium tungstate has contributes in the recovery of beta-pancreatic function of diabetic rat [11], and inhibits the gluconeogenesis metabolism [12], this study is also expected to provide the initial data for further applications of WO3 as an antidiabetic agent. This study was also evaluated the acute (LD50) and the sub acute toxicity of WO3 by orally administration of various doses of WO3 to rat for a day and 14 consecutive days. We also assessed the health effect of WO3 by determining the changes in body weight, biochemical blood changes, and histological changes in the liver and kidneys. The results found that rats given WO3 at 5000 mg kg-1 for 14 weeks exhibited changes in body weight and small deleterious effect on their liver and kidney. No biochemical changes were observed for creatinine and BUN parameter. In contrast, ALT parameter shows higher in the experiment than the control group. This information indicate that prolonged exposure to WO3 might toxic and cause abnormalities in the function of liver and kidney.

2. Experimental Method

2.1. Raw Material
Male rats of Sprague-Dawley strain (SD; from Faculty of Veterinary Medicine, Institut Pertanian Bogor, Indonesia), tungsten trioxide (WO3; prepared from ammonium tungstate pentahydrate; see reference [13], formaldehyde, absolute alcohol, xylol, paraffin, polyethyleneglycol (PEG), hematoxilin, and eosin. The rats were allowed water and standard laboratory chow ad libitum and were maintained under the standard light, temperature, and relative humidity conditions. The study protocol was approved by local ethical committee. All the experiments were performed in accordance with the guide for the care and use of laboratory animals. All chemicals were purchased from Wako Chemical Japan and used without further purification.

2.2. Preparation of Tungsten trioxide (WO3) micropowder
WO3 micropowder was prepared by heating ammonium tungstate(VI) pentahydrate (ATP; (NH4)2WO4·5H2O; Wako Chem. Co. Ltd., Japan) at 800°C. The decomposition of ATP into WO3 was confirmed by TG analysis [13].

2.3. Preparation of Tungsten trioxide (WO3) solution for LD50 (acute) and sub-acute assay
The tungsten trioxide solution (WO3) was prepared by dissolving a known weight of WO3 in polyethyleneglycol (PEG). The initial dose of WO3 for LD50 test was varied from 15, 45, 135, 405, to 1215 mg/kg. In the circumstance of no lethal animal found to the initial dose, the test was continued with higher concentration of WO3. The concentration of WO3 was varied from 2000, 4000, to 5000 mg/kg. The prepared dose was considered by the standard for drug candidate assay.

2.4. Oral Acute Toxicity Test
Forty-five of the test aclimated-animals were devided into nine group experiments animals. Test doses of WO3 were calculated in relation to the body weight of every animals; and administrated via oral gavage at 5000 mg/kg. The animals were regularly and individually observed for behavioral changes and general toxicity signs after dosing for the first 24 h, with special attention being given during the first 24 h. Thereafter, observation was continued daily for a total of 14 days [14].

2.5. Oral Sub-acute Toxicity Test
The experimental animals were devided into two groups of five rats each. The groups were treated daily with the LD50 dose which obtained from the acute test for 14 days. All treatments were
administered via oral gavage. The observation was continued for a total 15 days for preparing further hispatology analysis.

2.6. Biochemical Analysis and Histopathological Study
Clinical signs were observed a day during the 14-day treatment period. Body weights were measured once a day. On the 15th day, the animals were fasted overnight and blood samples collected via cardiac puncture. Vital body organs were dissected, cleansed of adhering tissues and rinsed in normal saline. The kidneys and livers were immediately stored in 10% of paraffin for histology. Paraffin sections were made and stained with hematoxylin and eosin for a through histopathological study [15][16]. For biochemical analysis purposes, the blood samples were centrifuged at 3000 rpm for 15 min. Diagnostic kits were used to evaluate these parameters, which included the alanine transaminase (ALT), creatinine, and Blood Urea Nitrogen (BUN). Histopathological examination was also conducted on the liver and kidney of the treated control groups [17][18]. Reference ranges for comparison are contingent on the method of analysis, animal species used and other experimental factors. Thus, in this study, the values obtained for the control group were considered as the reference values; and statistical analysis was conducted against the control group.

3. Results and Discussion
3.1. Acute Oral Toxicity Effect of WO₃ on Male Sprague Dawley (SD) Rats
There were no animal death in the first set of five groups of five male rats each receiving doses of 15, 45, 135, 405, and 1215 mg/kg of WO₃. No sign of toxicity was observed in the wellness parameters during the a day observation period. A similar observation was made in the second set of male rats treated with 2000, 4000, and 5000 mg/kg of WO₃. Therefore, the approximate acute lethal dose (LD₅₀) of WO₃ in male rats was estimated to be higher than 5000 mg/kg. Nonetheless, the knowledge gained from our toxicity study may serve for choosing more appropriately the test doses of WO₃ for chronic or sub chronic toxicity studies to report results of greater clinical relevance.

3.2. Sub-Acute (14 Days) Oral Toxicity Effect of Tungsten trioxide (WO₃) on Male Sprague Dawley (SD) Rats
The body weights and body weight gain of male rats treated with WO₃ at dose of 5000 mg/kg is presented in Fig.1. Both the control and experiment group showed increases in the body weight. It was observed higher increases of body weight gain of experiment group (8,14 g) compared to control group (5,04 g) at the 3rd days of observation compared with their initial weights. However, the statistic analysis of both groups (p<0.05) during 14 days observations show no significant differences were observed between control and experimental (treated) groups in their weight gain. This results indicate that no toxicity sign of WO₃ was observed after dosing for 14 days.
Figure 1. Initial and Daily Body Weight Measurements (g) of the Male Rats in the Sub-Acute Toxicity Study of Tungsten Trioxide (WO$_3$). Results were Expressed as the Mean ± S.E.M. of 5 Rats (Significantly Different from the Control; * p < 0.05; ** p < 0.01).

3.2.1. Effect of Oral Administration of Tungsten trioxide (WO$_3$) on Biochemical Parameters

3.2.1.1. Effect of Oral Administration of Tungsten trioxide (WO$_3$) on Biochemical Parameter of Alanine Transaminase (ALT) Profile

Biochemical test of alanine transaminase (ALT) shows the different average levels of ALT in control and treated group as can be seen in the Fig. 2. The ALT level of control group is 65.6, while the treated group was 1.6 times higher than that in the control group. This significantly increased of ALT indicate the abnormalities of liver function due to orally administrated with WO$_3$ for 14 days treatment.

Figure 2. Biochemical Parameter of ALT in the Serum of Male Rats Orally Treated with Tungsten Trioxide (WO$_3$). Results were Expressed as the Mean ± S.E.M. of 3 Rats.
3.2.1.2. Effect of Oral Administration of Tungsten trioxide (WO₃) on Creatinine Profile
As shown in the Fig. 3, creatinine levels in the control group is lower compared to the treatment group. However, both creatinine levels in the control and the treatment group are still in normal range, indicate the properly function of glomerulus to filter creatinine in the blood.

![Figure 3](image)

**Figure 3.** Biochemical Parameter of Creatinine in the Serum of Male Rats Orally Treated with Tungsten Trioxide (WO₃). Results were Expressed as the Mean ± S.E.M. of 3 Rats.

3.2.1.3. Effect of Oral Administration of Tungsten trioxide (WO₃) on Blood Urea Nitrogen (BUN) Profile
Similar to creatinine profile, the BUN level (Fig. 4) in the control group is also lower than that in the treatment group. Nevertheless, BUN levels for the control group and the treatment group are still in the normal range, which indicates the proper function of glomerulus in filtering urea in the blood.

![Figure 4](image)

**Figure 4.** Biochemical parameter of BUN in the serum of male rats orally treated with tungsten trioxide (WO₃). Results were expressed as the mean ± S.E.M. of 3 rats.

3.2.2. Histopathological Examination
Histopathological study was focus on to the liver and kidney due to the sensitivity of thus organs to harmful compounds and their potential to predict toxicity.

3.2.2.1. Effect of Oral Administration of Tungsten trioxide (WO₃) on Histopathological Feature of Liver
In the Fig. 5, it is shown the cross section of liver tissue in the control group and the treatment at dose of 5000 mg/kg after 15 days treatment. In was observed hidropis degeneration both in the control (shown by a purple arrow) and treated group (indicated by the arrow pink). In addition, many karyopiknosis were observed in the treated group as indicated by white arrow in the Fig. 5b.
Liver sections stained with hematoxylin and eosin (H & E-stained under 400x magnification showing the effect of tungsten trioxide (WO$_3$) in a 14-day sub-acute toxicity study in male rats: (a) Control group. Indicator: the portal vein (blue arrow), sinusoid (brown arrow), normal liver cells (green arrow), karyomegali (purple arrow), and biliary (arrowhead red); (b) treated group. Indicators: karyopiknosis (white arrow), and fatty degeneration (yellow arrow).

The hidropis degeneration was seen faded as cloudy, in which this was caused by the accumulation of fluid in the cells due to the decline in the balance of the cell that allows liquids to enter the cell cytoplasm swelling. In addition, fatty degeneration cytoplasm looks empty, caused by an excess of triglycerides in the cytoplasm. The accumulation of triglycerides in the liver may occur through several mechanisms: 1) inhibition of lipoprotein protein synthesis unit, 2) loss of potassium from hepatocytes resulted in the disruption of VLDL transfer through cell membranes, 3) deteritation of the lipid oxidation by mitochondria, and 4) the inhibition of the synthesis of phospholipids that are an important part of VLDL.

3.2.2.2. Effect of Oral Administration of Tungsten trioxide (WO$_3$) on Histopathological Feature of Kidney
The hidropis degeneration in control group was seem normal (Fig. 6). On the contrary, all rats in the treatment group exhibited a hidropis degeneration (shown by arrows pink). In addition, it was observed the small congestion in the tubular lumen of the treated group as shown by the white arrow and few deposit of protein in the proximal tubule (yellow arrow), indicate the damage of the tubules.

Figure 5. Liver sections stained with hematoxylin and eosin (H & E-stained under 400x magnification showing the effect of tungsten trioxide (WO$_3$) in a 14-day sub-acute toxicity study in male rats: (a) Control group. Indicator: the portal vein (blue arrow), sinusoid (brown arrow), normal liver cells (green arrow), karyomegali (purple arrow), and biliary (arrowhead red); (b) treated group. Indicators: karyopiknosis (white arrow), and fatty degeneration (yellow arrow).

Figure 6. Kidney sections stained with hematoxylin and eosin (H & E-stained under 400x magnification showing the effect of tungsten trioxide (WO$_3$) in a 14-day sub-acute toxicity study in male rats: (a) Control group. Indicator: the proximal tubules (blue arrow), the tubular lumen (white arrows), glomerular (brown arrow), Bowman's capsule (red arrow), and tubular epithelial cells (black arrow).
(b) treated group. Indicators: the congestion in the tubular lumen (white arrows), degeneration hidropis (pink arrows) and protein deposition in the tubular lumen (yellow arrow).

4. Conclusion
This study validated the toxic effect of tungsten trioxide (WO$_3$) at the dose of 5000 mg/kg with prolonged use. The toxic effects comprised changes of alanin transaminase (ALT) and the alteration in the normal physiological function of liver and kidney organs as an agents for harmful compound detoxifying. Prolonged use of WO$_3$ should be discouraged and lower doses encouraged.

5. Acknowledgements
This research supported by Direktorat Jendral Pendidikan Tinggi (Dirjen DIKTI) for Program Unggulan Perguruan Tinggi Negeri (PUPTN) and Graduate School of Universitas Pendidikan Indonesia

References
[1] Arutanti, O.; Nandiyanto, A.B.D.; Ogi, T.; Kim, T.O.; and Okuyama, K. 2015 ACS Appl. Mater. Interfaces. 7 (5) 3009
[2] Tajima, Y. 2003 J. Inorganic Biochem, 94,15
[3] Koutsospyros, A.; Braida, W.J.; Christodoulatos, C.; Dermatas, D.; Strigul, N.S. 2006 J. Hazard. Mater., 136 1
[4] Strigul N. S.; Agamemnon K.; Christos C. 2010 Ecotoxic. Environ. Safety., 73 164
[5] Erik L.; Wolf-Dieter S. 1999 Kluwer Academic, USA.
[6] Lee WJ. 2000, J Electron Mater. 29 183
[7] Arutanti, O.; Nandiyanto, A.B.D.; Ogi, T.; Iskandar, F.; Kim, T.O.; Okuyama, K. 2014 J. Alloys Compd. 591 121
[8] Arutanti, O.; Ogi, T.; Nandiyanto, A.B.D.; Iskandar, F.; Okuyama, K.; 2014 AIChE J. 60 41.
[9] Nandiyanto, A.B.D.; Arutanti, O.; Ogi, T.; Iskandar, F.; Kim, T.O.; Okuyama, K. 2013 Chem. Eng. Sci. 101 523
[10] Ogi T.; Sakamoto, Y.; Nandiyanto, A.B.D.; Okuyama, K. 2013 Ind. Eng. Chem. Res. 52 14441
[11] Gomez G.; Nuria R . M. M.; Benito C.; Jorge D.; Joan G.; Carmen C.; 2011 Talanta. 84 1011
[12] Kiersztan, A.; Winiarska, K.; Drozak, J.; Przedlacka M.; Wegrzynowicz M.; Fraczky, T.; and Bryla, J. 2004 Mol. Cell. Biochem. 30 1
[13] Nandiyanto, A.B.D.; Munawaroh, H.S.H.; Kurniawan, T.; and Mudzakir, A. 2016 Indones. J. Chem. 16(2) 124
[14] Nana, H.M.; Ngane, R.A.; Kuiate, J.R.; Mogtomo, L.M.; Tamokou, J.D.; Ndfor, F.; Mououkeu, R.S.; Etame, R.M.; Biyiti, L.; Zollo, P.H. 2011 J. Ethnopharmacol. 137 70
[15] Zepeda, R.J.; Candiracci, M.; Lobos, N.; Lux, S.; Miranda, H.F. 2014 Mar. Drugs. 12 5055
[16] Garrosa, M.; Jiménez, P.; Tejero, J.; Cabrero, P.; Cordoba-Diaz, D.; Quinto, E.J.; Gayoso, M.J.; Girbés, T. 2015 Toxins. 7 367
[17] Hussain, T.; Fareed, S.; Siddiqui, H.H.; Vijaykumar, M.; Rao, C.V. 2012 Asian Pac. J. Trop. Dis. 2 129
[18] Gupta, R.K.; Hussain, T.; Panigrahi, G.; Das, A.; Singh, G.N.; Sweety, K.; Faiyazuddin, M.; Rao, C.V. 2011 Asian Pac. J. Trop. Med. 4 964