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Evaluation of *in vivo* toxicity of rice husk used as fuel for cooking in households

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This study aimed to evaluate the toxicological effect of food cooked or water boiled with a fan-assisted top-lit-updraft rice husk fuelled gasifier stove named Paul Olivier 150 (PO150). Distilled water was boiled for 1 h using this stove in an opened pot and closed room. This water was then cooled to room temperature before being administered to rats with body weights ranging from 70 to 110 g. Two types of tests were performed: acute and sub-chronic toxicity test. For the acute toxicity study, a unique dose of 2 ml/100 g body weight (bw) of boiled water was administered orally to the rats. The animals were observed for toxic symptoms and mortality daily for 14 days. In a sub-chronic toxicity study, the boiled water, at doses of 0.5, 1 and 2 ml/100 g bw were orally administered daily for 28 days to rats. After these 28 days, the rats were sacrificed, blood samples were collected for haematological, biochemical and histological examination. The control rats were administered distilled water. The sample of distilled water boiled with rice husk fuelled gasifier stove showed no evidence of single dose toxicity (2 ml/100 g) when studying acute toxicity. For the sub-chronic toxicity study, boiled water at doses of 0.5, 1 and 2 ml/100 g showed significant difference in some parameters such as creatinine in males (71.81 mg/dL), uric acid (2.75 mg/dL) and total bilirubin (0.08 mg/dL), monocytes (0.49 10^3/µL) and granulocytes in females (2.70 10^3/µL) compared to the control group (64.16 mg/dL, 2.25 mg/dL, 0.19 mg/l, 0.37 10^3/µL, and 1.80 10^3/µL for each parameter respectively) but the data was below the threshold levels to be considered to have toxic effects. These showed that cooking in an open pot with a rice husk fuelled PO150 gasifier stove does not cause toxicity at the doses studied.

Key words: Acute toxicity, sub-chronic toxicity, biochemical analysis, hematological parameters, histopathology, rice husk.

INTRODUCTION

The adoption of fire so many years ago was surely one of the most powerful developments in human history. Fire for cooking has made the consumption of a much wider variety of foodstuffs and greatly enhanced food safety.

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Fire for heating has allowed humans to expand their zones of habitation to higher latitudes and elevations, and it has fundamentally transformed the patterns of social development. But with fire, also came the first anthropogenic pollution, evidenced by the soot still found in prehistoric caves (GEMS, 1990). Nearly 3 billion people worldwide, and a majority of households in developing countries, rely on solid fuels (such as wood, dung, crop residues, coal, and charcoal) with little or no access to modern fuels for cooking and other household energy needs (Lim et al., 2012; Smith et al., 2012). The types of fuel used for domestic needs such as cooking and heating can be categorized into non-solid and solid fuels (Torres et al., 2008).

Rice husk (solid fuel) constitutes about 20% of the weight of paddy and is composed of cellulose (50%), lignin (25-30%), silica (15-20%), and moisture (10-15%) (Bhupinder, 2018). According to the United States Department of Agriculture (USDA), paddy production in Sub-Saharan Africa (SSA) in 2018 was 26.5 million tons (IRRI RICESTAT, 2019). Based on paddy production estimates in 2018, some 5.3 million tons of rice husk were produced annually in SSA and this can be a perfect source of renewable energy. However, the husk in this region is mostly disposed by burning in the field or roads and/or dumping in river beds leading to high levels of land, water and air pollution. The rice husk can produce about 3000 kcal per kilogram of heat energy (Anderson et al., 2008). Combustion and gasification remain the most important viable options of using rice husk as fuel in SSA whereby the rice husk can be used unprocessed (Ndindeng et al., 2019) or processed into briquettes or pellets (Ndindeng et al., 2015).

Toxic health effects of solid fuels were identified as early as the late 18th century, when coal soot was recognized as a cause of scrotal cancer in chimney sweeps (Brown et al., 1957). In the 20th century, both coal and biomass fuels were subjects of intensive investigations on their possible negative health effects. The results of these researches suggest that some constituents of biomass smoke emissions have irritating, inflammatory and carcinogenic properties (Wei and Seow, 2012). Smoke emissions have carcinogenic and mutagenic properties in studies conducted on *in vitro* systems and animal models. At the population level, there is epidemiological evidence that biomass fuels are associated with respiratory and cardiovascular diseases such as lower respiratory tract infections, chronic obstructive lung disease and coronary heart disease (Dherani et al., 2008; Black et al., 2008). The incomplete combustion of these solid fuels results in much of the fuel energy being emitted as potentially toxic pollutants, including particles of varying sizes, carbon monoxide (CO), nitrogen dioxide, volatile and semivolatile organic compounds (e.g., formaldehyde and benzo[*a*]pyrene), methylene chloride, and dioxins (Naeher et al., 2007). The use of solid fuels, primarily for cooking, has been estimated to be responsible for >3.5 million premature deaths per year (plus an additional 0.5 million deaths from outdoor air pollution due to household fuel use) and 110 million disability-adjusted life years (DALYs) (Lim et al., 2012).

Ndindeng et al. (2019) evaluated five different rice husk top-lit updraft (TLUD) gasifier cook-stoves for use in cooking operations in Africa. This study demonstrated that fan-assisted cook-stoves especially PO150 recorded better thermal and emission indices and are safer to use than the natural draft gasifier stove (Mayon). Although PO150 operator may safely use the stove for cooking in well ventilated environments, questions still exist as to whether some compounds emitted by the stove can contaminate the food being cooked and exert toxic effects on consumers. However, to our knowledge, no study on the *in vivo* toxicity of rice husk as a fuel has been described in the literature. Therefore, in the present investigation, we aimed to investigate the toxicity (both oral acute and sub-chronic) of distilled water boiled on P0150 gasifier using rice husk as fuel on rats.

MATERIALS AND METHODS

Operation of the stove and preparation of the water sample

PO150 gasifier operates on batch mode and on a forced-draft system which is a fan with a fueling capacity of 0.78 kg per batch and a batch lasts for 30 min. When the rice husk in the gasifier was completely used up, the biochar was discarded before new fuel was put into the gasifier to initiate another energy generation process. In order to reduce the fuel refilling time and ensure smooth cooking of dishes whose cooking time is higher than the maximum burning time for batch type gasifiers, stove switching was used as previously described (Ndindeng et al., 2019). Briefly, two PO150 gasifiers, A1 and A2 were produced and used for the study. When A1 was running, A2 was filled with rice husk and placed nearby. The gasifier A2 was lit when the burning time in A1 was 5 min to stopping time. When A1 stopped, the pot was transferred to A2 and the biochar in A1 chamber was discarded, new fuel filled and placed nearby.

Distilled water was boiled every day during the period of the experiment (28 days). The fan-assisted gasifier PO150 (Figure 1) was placed in a room with window and door closed.

Five liters of distilled water were put in an open pot with a capacity of 10 L. Since the cooking time of food varies (10 min to 2 h), the time the water is kept boiling on the stove is critical and the longer the time, the better, to allow for any possible toxins from the stove emissions to dissolve in the water; the water was therefore allowed to boil for about 1 h from the boiling point on the stove using the rice husk as fuel. This water was then cooled to room temperature before being administered to the rats.

Experimental animals

For the evaluation of rice husk-related toxicity, 48 albino rats of *Wistar* breed (20 males and 28 non-pregnant females), aged about 6 weeks, and body weights ranging from 70 to 110 g at the beginning of the experiment, were used. They were purchased from the Animal House of the Laboratory of Animal Physiology, Department of Biochemistry, University of Yaoundé I, and bred at

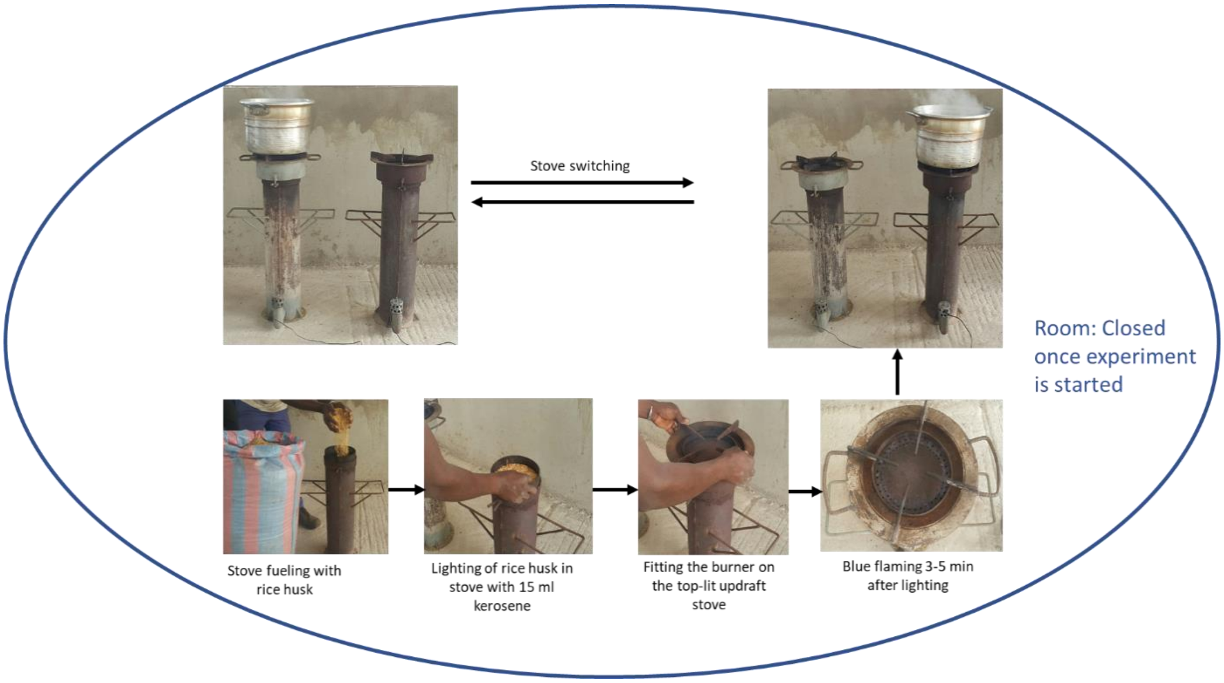

Figure 1. Schematic presentation of the setup for boiling water with a PO150 fan-assisted rice husk fuelled gasifier stove.

room temperature for a 12 h light/dark photoperiod cycle. A seven (7)-day adaptation period was observed before the experiment. They were kept in their plastic cages where they received the standard diet and water *ad libitum*. The litter used was sawdust, renewed twice per week to ensure good hygienic status of the animals. Authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Reg. N° FWA-IRD 0001954).

Grouping of animals

The 48 *Wistar* albino rats were randomly divided into 6 groups of 8 animals each. Group 1 (consisting of 8 females) was used for the acute toxicity assessment. Female rats were used because literature surveys of conventional LD50 tests show that, although there is little difference in sensitivity between the sexes, in cases where differences are observed, females are generally slightly more sensitive (OCDE, 2001). And the other 5 groups (8 rats per group, made up of 4 females and 4 males) were used for the sub-chronic toxicity study.

Acute toxicity study in rats

For acute toxicity testing, in rodents, the volume of administered substance should not normally exceed 1 mL/100 g of body weight. However, for aqueous solutions, 2 mL/100 g body weight (bw) can be considered. In this study, distilled water boiled with rice husk was given to the rats at the unique dose of 2 ml/100 g bw, according to the Organization for Economic Cooperation Development (OECD) guidelines 423 (OCDE, 2001). Eight healthy *Wistar* female rats were randomly divided into 2 groups (4 females per group). On the eve of the day of the experiment, food but not water was withheld overnight. Group 1 (Normal control group) received distilled water, given orally. Group 2 (The experimental group) orally received a unique dose of 2 ml/100 g bw distilled water that was boiled with rice husk. Food was withheld for a further 4 h after giving the water. The rats were observed individually for general behaviour, toxic symptoms and mortality during the first 30 min. This observation continued every 30 min, during the first 4 h after administration of the unique dose of 2 ml/100 g bw boiled water, the animals were weighed every 2 days, for a total of 14 days and during this period, signs of toxicity including changes in hairs, motility, tremors, mass, grooming, sensitivity to noise after metal shock, stool appearance, mobility and death were observed. The rats were sacrificed by cervical dislocation, and their organs were excised (heart, liver, spleen, lungs, kidneys), and weighed using an analytical balance.

Sub-chronic toxicity study in rats

The sub-chronic toxicity study was carried out on the rats according to the Organization for Economic Cooperation and Development (OECD guideline 407 for testing of chemicals on sub-chronic toxicity with slight modifications); which stated that the volume given to rats should not normally exceed 1 mL/100 g of body weight, however in the case of aqueous solutions 2 mL/100 g body weight can be considered (OECD, 2008). Forty healthy *Wistar* rats were weighed, orderly marked, and randomly divided into 5 groups (4 males and 4 females per group). Group 1 (Control group) received distilled water by oral gavage throughout the course of the study. The experimental groups (2-4) were orally administered samples of distilled water boiled with rice husk stove as follows: Low dose (0.5 ml/100 g), medium dose (1 ml/100 g) and high dose (2 ml/100 g) body weight/day, respectively, for 28 days. The body weight was measured every 2 days and signs of toxicity were noted daily. At the end of these 28 days, groups 1 to 4 were sacrificed while the physiological condition of the rats of group 5 was restored for another 2 weeks (with food and water supplied *ad libitum*). Group 5 was orally administered samples of a high dose of boiled distilled water (2 ml/100 g) for 28 days but not sacrificed at the end of 28 days as groups 1 to 4. Group 5 was observed for an additional 14 days and sacrificed on the 42nd day. All the rats survived and were anesthetized with carbon dioxide and blood samples were obtained from the eyes of the rats using capillary tubes for hematological and serum biochemical studies. After blood collection, the rats were sacrificed by cervical dislocation.

Measurement of body and organ weights

The animals were weighed every 2 days and the percentage weight gain (%) was calculated using the formula:

$$\text{Weight gain (\%)} = \frac{W_f - W_i}{W_i} \times 100$$

where W_f is the final weight and W_i is the initial weight.

All the animals in this study were subjected to general autopsy. Animals were pinned down in a dissection tray by placing them with ventral side up. The abdominal skin was lifted with forceps and cut through with scissors. The scissor was inserted under the skin and moved towards the cephalic direction. The rats were cut along the body midline, from the public region to the lower jaw. A lateral cut was made about halfway down the ventral surface of each limb. The liver, heart and kidneys were removed, cleaned, and kept in the refrigerator. The relative weight of the liver, heart and kidneys was determined by the formula:

$$\text{Relative organ weight} = \frac{weight\ of\ organ}{Animal\ weight} \times 100$$

Biochemical parameters

Blood samples were collected in non heparinized tubes and centrifuged at 3000 rpm to obtain the serum that served for the assessment of the parameters for liver and kidney functions. The experiment was performed in accordance with protocols provided with commercial kits, Fortress Diagnostics, reviewed in October 2007. The levels of aspartate amino transferase (AST), alanine amino transferase (ALT), creatinine, uric acid, total bilirubin and proteins were assessed. Creatinine, uric acid and total bilirubin and proteins were analyzed using the method described by Timothy et al. (2015). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were assayed using the colorimetric test of Reitman and Frankel (1957) as published by Rodier and Mallein (1983).

Hematological analysis

The following haematological parameters were evaluated with the help of a "Hospitex Diagnostics Hema Screen 18" Automated Analyzer from the Haematology Laboratory of the Yaoundé Central Hospital: white blood cell count (WBC), haemoglobin (Hb), red blood cell counts (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), hematocrit (HCT), lymphocytes (LYM), monocytes (MON), granulocytes (GRA) and platelet count (PLT).

Histopathological study

The liver, heart and kidneys stored in formalin 10% for 3 weeks were cut into small pieces of 5 to 10 mm. The tissues were dehydrated in an ascending series of alcohol, soaked in xylene, and embedded in paraffin wax melted at 60°C. Serial sections (5 µm thick) obtained by cutting the embedded tissue with microtome were mounted on 3-aminopropyl triethsilane coated slides and dried for 24 h at 37°C (Baravalle et al., 2006). The sections on the slides were deparaffinised with xylene and hydrated in a descending series of alcohol. They were then stained with Mayer's haematoxylin and eosin dyes, dried and mounted on a light microscope (X100 and X200) for histopathological examination.

Statistical analysis

The data was analyzed using the software, Excel and Graph Pad. Quantitative data were presented as mean ± standard deviation (SD) on graphs and tables. One-way Analysis of Variance (ANOVA) was used to compare the means between the groups. This was accompanied by the post hoc Tukey's multiple comparison test to determine significant differences between values. The value of $p < 0.05$ was considered statistically significant.

RESULTS

Acute oral toxicity test

In all the eight female animals used for the test, no signs of toxicity or death were observed among the rats during the 14 days of the acute toxicity experimental period, after the administration of a single oral dose of 2 ml/100 g of distilled water, boiled with rice husk as fuel. The average gain in body weight of the rats was 10.2 ± 2.32, 40.75 ± 7.78 and 60.75 ± 8.78% for days 2, 8 and 14, respectively. The body weight gradually increased within the normal range of body weight gain. After 14 days of testing, all the rats were subjected to gross necropsy. The pathological studies on the liver and kidneys of the rats tested showed no significant abnormal changes in colour, size, shape and texture compared to the control. These results suggest that water boiled with rice husk as fuel was not toxic, after an acute exposure.

28-Day sub-chronic oral toxicity study

Effects on the behaviour, organ and body weight

The administration of various doses of water boiled with rice husk as fuel (0.5, 1 and 2 ml/100 g bw) for 28 days, had no significant change ($p>0.05$) on the body weight of either male or female rats compared to the controls (Figure 2A and B). No deaths or obvious clinical signs of toxicity in the rats were observed in all the groups, including the group that received the highest dose of 2 ml/100 g bw. Figure 3A and B show the relative organ weights of the male and female rats after 28 days of administration of the boiled water sample. The relative organ weights of the liver and kidneys, heart, lungs and spleen evaluated and calculated at necropsy in the treated groups did not show any significant difference (p

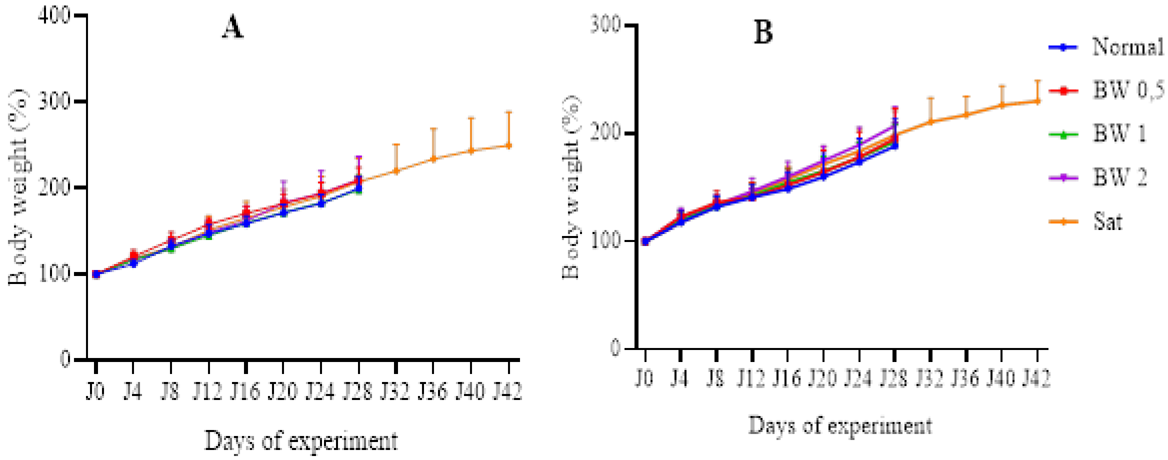

Figure 2. Effect of water boiled with rice husk on the body weights of male (A) and female (B) wistar rats. n = 4 corresponding to number of rats per sex. Each value represents mean ± SD. Control: healthy rats that received distilled water; Sat: Rats that received 2 ml/100 g bw of boiled water and observed 14 days more, after the end of the experiment; BW0.5, BW1 and BW 2 represent groups of rats that received boiled water at doses of 0.5 ml/100 g, 1 ml/100 g and 2 ml/100 g bw, respectively.

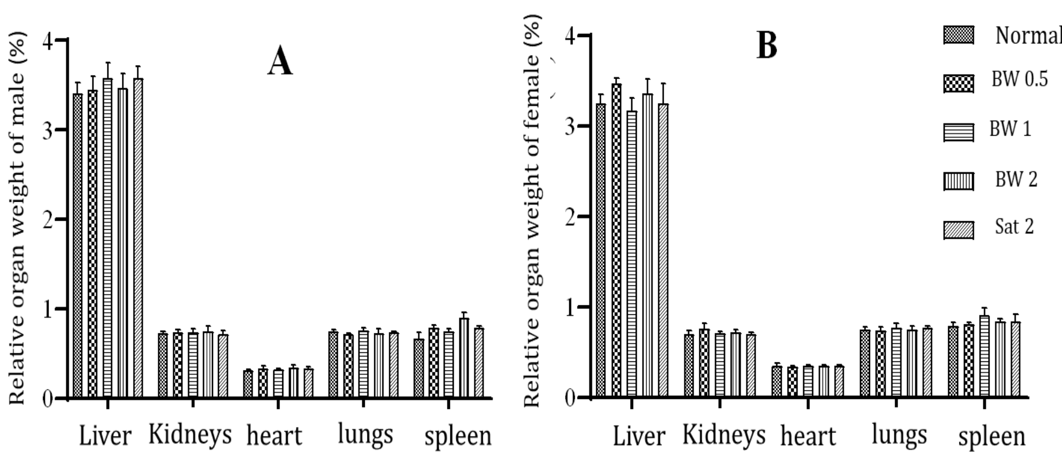

Figure 2. Relative organ weights of male (A) and female (B) rats that received different doses of the boiled water for 28 days. n = 4 corresponding to number of rats per sex. Each bar represents mean ± SD. Control: healthy rats that received distilled water; Sat: Rats that received 2 ml/100 g bw of boiled water and observed 14 days more, after the end of the experiment, BW0.5, BW1 and BW 2 represent groups of rats that received boiled water at doses of 0.5 ml/100 g, 1 ml/100 g and 2 ml/100 g bw, respectively.

> 0.05) from those of the control.

Effects on biochemical and haematological parameters

The results of the biochemical parameters are shown in Table 1. The results for male rats showed no significant difference in some parameters (ALT, AST, Total protein, urea and Total bilirubin) at all treatment doses except creatinine which significantly (P<0.05) increased at the dose of 1 ml/100 g compared to the control.

In the female rats (Table 2), uric acid and total bilirubin recorded significant differences, at the dose of 2 ml/100 g administered. The analyses showed significant increase (P<0.05) in uric acid while total bilirubin significantly decreased, with more decrease 14 days after the end of the experiment compared to the control group.

The effect of the boiled water sample on the haematological indices of the rats was examined at the end of the experiment (Tables 3 and 4). Analysis of variances showed no significant difference in most of the parameters except the platelets (P<0.05), compared to the control in the male rats. However, in the female rats,

Table 1. Biochemical profile of male rats that received different doses of boiled water for 28 days.

Parameter	Groups				
	Control	0.5 ml/100 g	1 ml/100 g	2 ml/100 g	Satellite (2 ml/ 100 g)
AST (UI/L)	134.44 ± 7.877	144.13 ± 10.24	136.37 ± 2.59	144.40 ± 5.89	134.17 ± 4.67
ALT (UI/L)	36.75 ± 2.40	43.06 ± 2.36	41.08 ± 6.04	38.07 ± 10.22	40.70 ± 2.84
Creatinine (mg/dL)	64.16 ± 2.43	65.93 ± 1.93	71.81 ± 1.09*	67.28 ± 1.72	63.89 ± 1.86
Uric acid (mg/dL)	2.44 ± 0.10	2.24 ± 0.07	2.2 ± 0.16	2.13 ± 0.10	2.11 ± 0.18
Protein (mg/dL)	2.17 ± 0.05	2.17 ± 0.05	2.28 ± 0.03	2.22 ± 0.06	2.17 ± 0.10
Bilirubin (mg/l)	0.19 ± 0.01	0.19 ± 0.03	0.23 ± 0.04	0.26 ± 0.01	0.18 ± 0.02

Table i. Biochemical profile of female rats that received different doses of boiled water for 28 days.

Parameter	Groups				
	Control	0.5 ml/100 g	1 ml/100 g	2 ml/ 100 g	Satellite (2 ml/100 g)
AST (UI/L)	171.10 ± 4.95	154.85 ± 9.05	147.08 ± 7.68	143.64 ± 11.00	160.98 ± 2.65
ALT (UI/L)	52.77 ± 4.43	50.35 ± 7.95	42.82 ± 2.14	44.51 ± 3.26	48.17 ± 3.77
Creatinine (mg/dL)	67.55 ± 0.39	72.01 ± 2.86	65.59 ± 4.43	69.11 ± 3.65	66.01 ± 1.24
Uric acid (mg/dL)	2.25 ± 0.19	2.45 ± 0.09	2.29 ± 0.17	2.75 ± 0.13*	2.27 ± 0.15
Protein (mg/dL)	2.23 ± 0.03	2.22 ± 0.12	2.06 ± 0.05	2.23 ± 0.07	2.17 ± 0.06
Bilirubin (mg/l)	0.19 ± 0.01	0.19 ± 0.03	0.15 ± 0.03	0.08 ± 0.02*	0.10 ± 0.04*

Values are expressed as mean ± SD; *significantly different from the control group ($p < 0.05$). AST: Aspartate amino transferase, ALT: alanine amino transferase, n = 4 corresponding to number of rats per sex. Control: healthy rats given distilled water; Sat: Rats that received 2 ml/100 g of boiled water and observed 14 days more, after the end of the experiment, BW 0.5, BW1 and BW 2 represent groups of rats that received boiled water at doses of 0.5 ml/100 g, 1 ml/100 g and 2 ml/100 g bw respectively.

Table 3. Hematological profile of male rats given different doses of boiled water for 28 days.

Parameter	Control	0.5 ml/100 g	1 ml/100 g	2 ml/100 g	Satellite (2 ml/100 g)
Red blood cell (10^6/µL)	4.58±0.28	4.66±0.19	4.23±0.42	5.01±0.37	4.57±0.24
Haemoglobin (g/dL)	15.50±0.34	15.53±0.48	15.77±0.34	13.70±0.82	14.67±0.54
Hematocrit (%)	49,63±1,33	41.30±0.73	49.77±1.56	43.07±1.01	47.03±0.48
MCV (fL)	88.33±0.68	86.67±0.68	88.33±0.26	85.33±2.46	86.00±1.55
MCH (pg)	31.10±1.70	34.83±0.79	32.13±1.01	27.30±1.22	32.17±0.92
MCHC (g/dL)	31.87±0.16	33.93±0.61	33.70±1.28	32.70±0.95	32.90±0.99
White blood cell (10^3/µL)	7.97±0.14	7.93±0.25	8.30±0.35	8.83±0.96	7.93±0.29
Lymphocytes (10^3/µL)	2.63±0.36	3.73±0.52	3.57±0.29	4.77±1.12	4,00±0.65
Monocytes (10^3/µL)	0.25±0.05	0.37±0.20	0.30±0.18	0.27±1.31	0.37±0.05
Granulocytes (10^3/µL)	3.20±0.08	3.47±0.21	4.37±0.72	4.23±0.36	3.30±0.97
Platelets (10^3/µL)	356.0±19.3	360.7±4.5	362.3±29.4	446.3 ±3.2*	368.0±5.5

there was no significant difference on several parameters such as red blood cell counts, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell counts, lymphocytes, and platelet. On the other hand, for monocytes the analyses showed a significant increase at the 2 ml/100 g dose ($P<0.05$). The number of granulocytes also increased significantly ($P<0.05$) at all doses when compared with the control.

Histopathology study

Figures 4 and 5 show the histopathology profile of male and female rats, respectively after 28 days of administration of the water sample. Light microscopic examination of sections of the kidney, liver, and heart of

Table 4. Hematological profile of female rats given different doses of boiled water for 28 days.

Parameter	Control	0.5 ml/100 g	1 ml/100 g	2 ml/100 g	Satellite (2 ml/100 g)
Red blood cell (10^6/µL)	4.48±0.21	4.56±0.39	4.40±0.26	4.25±0.12	4.15±0.11
Haemoglobin (g/dL)	14.80±0.12	15.10±0.28	13.67±0.39	14.17±0.54	12.57±0.38
Hematocrit (%)	42.87±1.27	43.47±3.45	41.63±0.72	45.70±1.20	40.30±0.13
MCV (fL)	86.00±0.89	86.00±1.18	87.00±1.34	88.00±2.37	87.67±0.68
MCH (pg)	30.07±0.52	31.33±0.52	33.03±3.07	32.00±1.72	29.90±0.45
MCHC (g/dL)	31.83±0.68	32.40±1.95	32.23±1.19	31.10±0.85	31.90±0.18
White blood cell (10^3/µL)	7.80±0.20	7.77±0.23	7.63±0.09	8.33±0.35	7.95±0.31
Lymphocytes (10^3/µL)	2.07±0.49	2.87±0.42	3.13±0.63	2.97±0.23	2.50±0.24
Monocytes (10^3/µL)	0.37±0.14	0.32±0.08	0.30±0.11	0.49±0.31*	0.39±0.09
Granulocytes (10^3/µL)	1.80±0.29	2.70±0.76*	2.53±0.07 *	2.47±0.30*	1.93±0.11
Platelets (10^3/µL)	393.7±22.76	455.7±21.99	341.3±3.36	436.0±24.19	399.3±56.66

Values are expressed as mean ± SD; * and ** = significantly different from the control group ($p < 0.05$ and $p < 0.001$, respectively). MCV: Mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, WBC: white blood cell, n = 4 corresponding to number of rats per sex. Control : Healthy rats given distilled water; Sat: Rats that received 2 ml/100 g of boiled water and observed 14 days more, after the end of the experiment, BW0.5, BW1 and BW 2 represent groups of rats that received boiled water at doses of 0.5 ml/100 g, 1 ml/100 g and 2 ml/100 g bw, respectively.

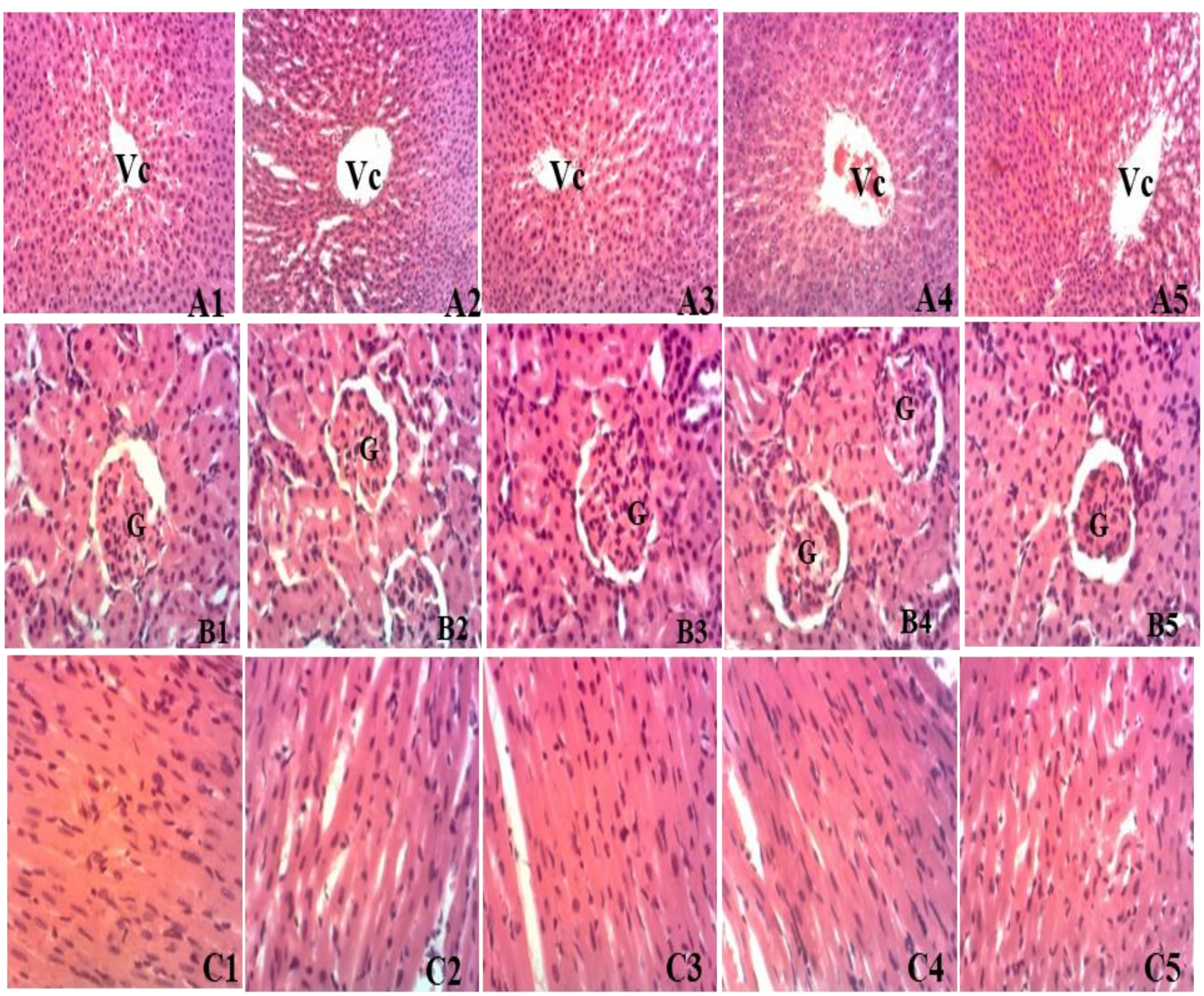

Figure 3 . Histopathological examination of organs of male rats in a sub-chronic oral toxicity study. (A-C) the liver, kidney, and heart, respectively; and (1-5) the control, the low, middle, high dose and the satellite (highest) dose groups, respectively. G, Glomerulus; CV, Central Vein.

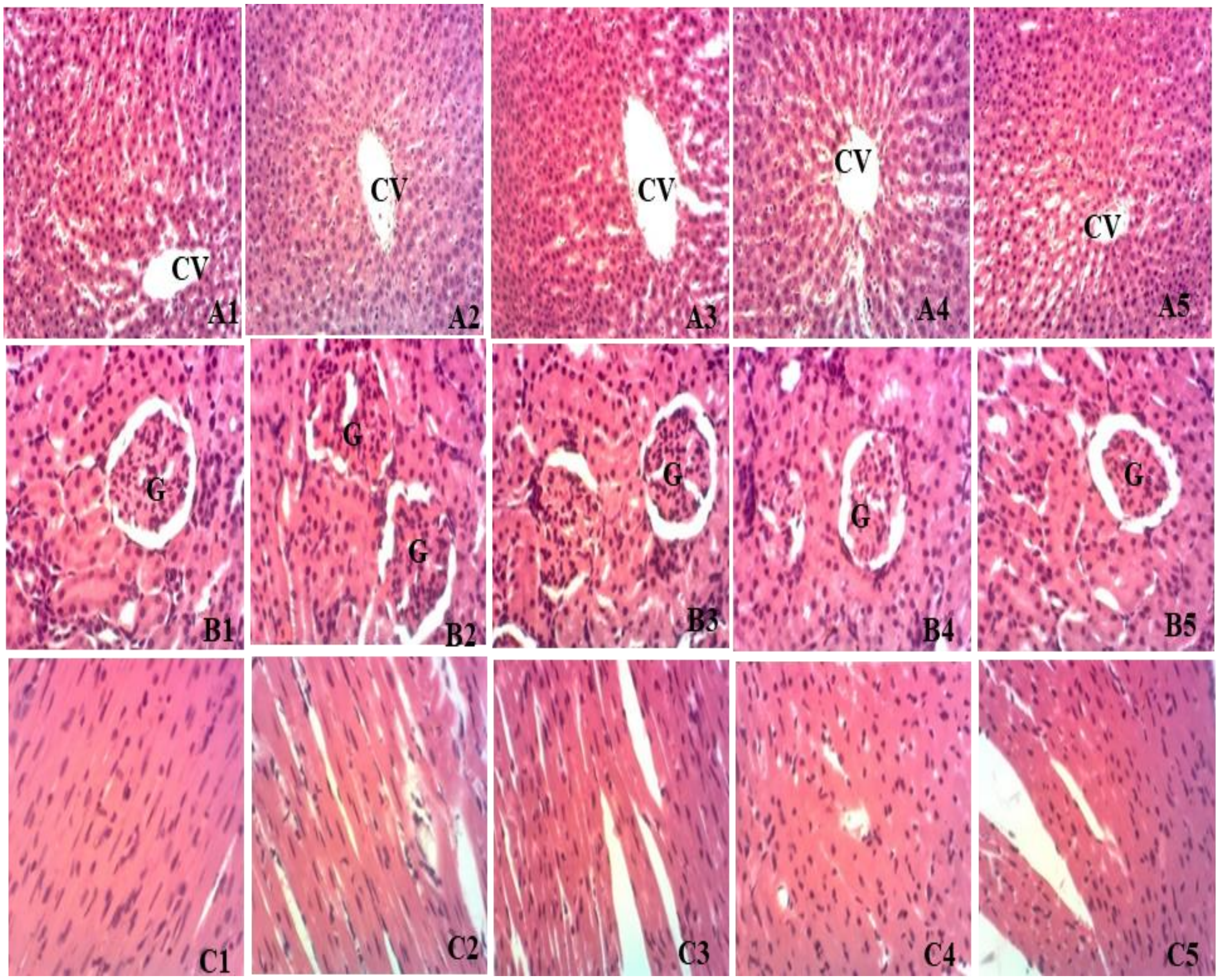

Figure 4. Histopathological examination of organs of female rats in a sub-chronic oral toxicity study. (A-C) the liver, kidney, and heart respectively; and (1-5) the control, the low, middle high dose and the satellite (highest) dose groups, respectively. G, Glomerulus; CV, Central Vein.

rats from the control group and those given a low dose (0.5 ml/100 g bw), medium dose (1 ml/100 g bw) and high dose (2 ml/100 g bw), showed a normal histology.

DISCUSSION

As an initial step, an oral acute toxicity study was conducted. It was observed that irrespective of the sex and the treatment, an increase in weight of the same amplitude was observed with no statistical differences ($p<0.05$) between the test groups. Weight increase is an indication of growth. The reasonable homogeneous increasing trend of body and organ weight in all the groups of rats can be taken as an indication of the low effect of the different treatments on the animals' feeding and health. Njayou et al. (2010) observed that body weight may increase or decrease in relation to the sex, absence of toxicity or induced anorexia. The results show that there was no mortality or abnormal behaviour or signs of toxicity after administration of the highest dose (2 ml/100 g bw) for up to 14 days. This shows that, according to the labeling and classification of acute systemic toxicity recommended by the OECD, the lethal dose may be above this dose limit, known as Class 5 status (OECD, 2001). Further investigation was conducted to evaluate the sub-chronic toxicity of rice husk boiled water for 28 days in rats.

Previous studies have shown that substances administered in chronic disease conditions may need toxicological evaluation of repeated doses (sub-chronic toxicity study), since daily use may result in accumulation in the body with gradual effects on tissues and organs (Abotsi et al., 2011; Bariweni et al., 2018). In this study, 28 days of oral toxicity evaluation of water boiled with rice husks, at doses of 0.5, 1 and 2 ml/100 g bw, did not show any adverse clinical signs or negative influences on behaviour and mortality in the test groups. Changes in feed and water intake and body weight gain have been used as an indicator of the general health status of experimental animals (El Hilaly et al., 2004). Feed consumption is regulated though several complex biological mechanisms can ensure relatively constant body weight over long periods of time (Kuriyan et al., 2007). No significant difference in body weight gain of the male and female rats compared to the control was recorded. Again, in toxicity studies, changes in the weight of organs are sensitive indicators of toxicity, effects on enzymes, physiologic disturbances and target organ injury (Michael et al., 2007). An increase in organ weight suggests the occurrence of hypertrophy while a decrease suggests necrosis in the target organ (Teo et al., 2002). In this study, the relative organ weight of the liver and kidneys, heart, lungs and spleen evaluated in the test groups did not show a significant difference in both sexes at all doses compared to the control.

The study of biochemical parameters is an indicator of toxicity, raising the effectiveness or the installation of toxicity on vital organs. In this study, parameters like creatinine in male, total bilirubin, and uric acid in female rats showed significant differences between the test groups and controls. The dose of 1 ml/100 g in males significantly increased creatinine and at 2 ml/100 g bw administered in females, uric acid was also significantly increased ($P<0.05$) but total bilirubin significantly decreased. Creatinine is an excretion product of muscle activity, which circulates in blood. Its elimination is exclusively renal, so there is a correlation between creatinine levels and renal function. Most creatinine that is eliminated by the kidneys is freely filtered in renal glomeruli, and a small fraction is filtered by the tubular component, which is a good indicator of renal-glomerular function (Bohinski, 1991; Raju et al., 2016; Ghorbel et al., 2016; Belhadj et al., 2018). The decrease of these parameters would show the hepatoprotective action of boiled water sample at this dose. Uric acid is a waste product of nucleic acid metabolism (Wallace, 2004). It is formed by the liver and mainly excreted by the kidneys (65-75%) and intestines (25-35%) (Alvarez et al., 2010). In the present study, blood uric acid was high in female rats (2 ml/100 g), suggesting kidney malfunction (Raju et al., 2015, 2016; et al., 2018). The functioning of the liver was assessed by the serum total protein, bilirubin and albumin. An increase in these parameters is usually seen in cancerous conditions, or following high protein diet (Tietz et al., 1994). Our study showed a significant decrease in total bilirubin in the female rats at the dose of 2 ml/100 g suggesting the toxic effect of the boiled water on the liver of the animals. The total protein serum level did not differ significantly from the control group. This shows that the effect on the liver could be a mild toxic effect affecting only the female rats. Generally, it appeared that the water affected the females at a lower dose than the males, which were not affected.

Haematopoiesis is the process of blood cell formation. Analysis of the hematological parameters is important in assessing the toxic effects of test substances, as well as in determining the physiological and pathological status of the body, as variations in these parameters may indicate toxicity associated with the test substances and various diseases and conditions, including anaemia, leukaemia, reactions to inflammation and infections (Olson et al., 2000; Martini et al., 2012). There was no significant difference in several parameters such as red blood cell counts, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell counts, lymphocytes, and platelets between the treated groups and the control group, indicating that the boiled water had no effect on the circulating blood cells of the tested animals. For monocytes, the analyses showed a significant increase at the dose of 2 ml/100 g, the number of granulocytes also significantly increased ($P<0.05$) at all the doses when compared with the control in females. However, these differences obtained in this study do not show a hematological change, since they are within the normal range of these parameters for good health in this animal species (Giknis et al., 2008).

These differences obtained between the tested animals and the control could be explained by the presence of suspended matter such as the rice husk ash (RHA) in this boiled water. This RHA is found in the boiled water through the ventilation by the fan. According to Xu et al. (2012), ash has the highest proportion of silica content among all plant residues. The average composition of well-burnt RHA is 90% amorphous silica. The findings of this study are in line with those of Wai et al. (2017) who investigated the *in vivo* toxicity of Silica nanoparticles (SiNPs) of 150 nm in various dosages via intravenous administration in mice and showed that SiNPs were biocompatible and safe for *in vivo* use in mice.

The histology of the kidneys, liver and heart in the male and female rats did not produce any toxic changes, despite presenting some changes in biochemical tests; the histological study suggests the safety of the rice husk boiled water in these organs. This shows that rice husk used as fuel in PO150 gasifier stove is non-toxic, and therefore safe for cooking food.

Conclusion

The results obtained in this work suggested that rice husk used as fuel is not toxic at all the doses studied (0.5, 1 and 2 ml/100 g bw) and did not produce any evident symptoms in the acute and sub-chronic oral toxicity studies in both male and female rats. The histological examination revealed no changes in the internal organs, like kidneys, liver and heart of the rats, in both the control and test groups. However, more studies are required to evaluate the safety of using rice husk for a longer period of time.

CONFICT OF INTERESTS

The authors have not declared any conflict of interests.

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