Vol. 14(2), pp. 46-56, April-June 2020
DOI: 10.5897/AJBR2020.1078
Article Number: D904F4C63565
ISSN 1996-0778

http://www.academicjournals.org/AJBR

ACADEMIC JOURNALS

African Journal of Biochemistry Research

Full Length Research Paper

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

Mbassi Josiane Emilie Germaine[1,3*], Sali Atanga Ndindeng[2], Achu Mercy Bih Loh[3], Dimo Théophile[4] and Mbacham Fon Wilfred[3]

[1]Food Technology Laboratory, Institute of Agricultural Research for Development (IRAD), P. O. Box 2123 Yaoundé, Cameroon.
[2]Africa Rice Center, M'bé Research Station, Bouake, 01BP 2551, Côte d'Ivoire.
[3]Department of Biochemistry, Faculty of Sciences, University of Yaoundé I, P. O. Box 812, Cameroon.
[4]Laboratory of Animal Physiology, Department of Animal Biology, University of Yaoundé I- P. O. Box 812, Cameroon.

Received 18 January, 2020; Accepted 19 March, 2020

This study meant to assess the toxicological impact of nourishment cooked or water overflowed with a fan-helped top-lit-updraft rice husk fuelled gasifier stove named Paul Olivier 150 (PO150). Refined water was bubbled for 1 h utilizing this stove in an opened pot and shut room. This water was then cooled to room temperature before being managed to the rodents with body loads going from 70 to 110 g. Two kinds of tests were performed: Acute and sub-chronic toxicity tests. For the acute toxicity study, an extraordinary portion of 2 ml/100 g body weight (BW) of bubbled water was managed orally to the rodents. The creatures were watched for harmful indications and mortality day by day for 14 days. In a sub-chronic toxicity study, the bubbled water, at dosages of 0.5, 1 and 2 ml/100 g BW were orally managed day by day for 28 days to rodents. Following 28 days, the rodents were yielded, Blood tests were gathered for hematological, biochemical and histological assessment. The control rodents were managed in refined water. The example of refined water overflowed with rice husk fuelled gasifier stove indicated no proof of single-portion toxicity (2 ml/100g) when studying acute toxicity. For the sub-chronic toxicity study, bubbled water at dosages of 0.5, 1 and 2 ml/100 g indicated huge contrast in certain parameters, for example, creatinine in guys (71.81 mg/dL), uric corrosive (2.75 mg/dL) and complete bilirubin (0.08 mg/dL), monocytes (0.49 103/µL)and granulocytes in females (2.70 103/µL) contrasted with the control gathering (64.16 mg/dL, 2.25 mg/dL, 0.19 mg/l, 0.37 103/µL and 1.80 103/µL for every parameter separately) however, the information did not ascend to the level for the responses to be viewed as a poisonous impact. These demonstrated that cooking in an open pot with a rice husk fuelled PO150 gasifier stove doesnot cause toxicity at the dosages considered.

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

INTRODUCTION

The adoption of fire in such a large number of years before was without a doubt one of the most remarkable

*Corresponding author. E-mail: josianembassi@yahoo.fr.

advancements in mankind's history. Fire for cooking has made the utilization of a lot more extensive assortment of staples and incredibly improved sanitation. Fire for warming has permitted people to grow their zones of home to higher scopes and rises, and it has in a general sense changed the examples of social advancement. In any case, with fire additionally came the main anthropogenic contamination, proven by the ash despite everything found in ancient caverns (GEMS, 1990). About 3 billion individuals around the world, and a dominant part of families in creating nations, depend on strong powers, (for example, wood, waste, crop deposits, coal, and charcoal) with almost no entrance to current fills for cooking and other family unit vitality needs (Lim et al., 2012; Smith et al., 2012). The kinds of fuel utilized for household needs, for example, cooking and warming can be ordered into non-strong and strong energizes (Torres et al., 2008).

Rice husk (strong fuel) establishes about 20% of the heaviness of paddy and is made out of cellulose (half), lignin (25-30%), silica (15-20%), and dampness (10-15%) (Bhupinder, 2018). As per the United States Department of Agriculture (USDA), paddy creation in Sub-Sahara Africa (SSA) in 2018 was 26.5 million tons (IRRI RICESTAT, 2019). In view of paddy creation evaluations in 2018, some 5.3 million tons of rice husk was delivered yearly in SSA and this can be an ideal wellspring of the sustainable power source. Be that as it may, the husk right now for the most part discarded by copying in the field of streets or potentially dumping in waterway beds prompting significant levels of land, water, and air contamination. The rice husk can deliver around 3000 kcal per kilogram of warmth vitality (Anderson et al., 2008). Burning and gasification remain the most significant feasible alternatives of utilizing rice husk as fuel in SSA whereby, the rice husk can be utilized natural (Ndindeng et al., 2019) or prepared into briquettes or pellets (Ndindeng et al., 2015).

Poisonous wellbeing impacts of strong powers were distinguished as ahead of schedule as the late eighteenth century when coal residue was perceived as a reason for scrotal disease in fireplace clears (Brown et al., 1957). In the twentieth century, both coal and biomass powers were subjects of escalated examinations on their conceivable negative wellbeing impacts. The consequences of these examine recommend that a few constituents of biomass smoke emanations have aggravating, incendiary and cancer-causing properties (Wei-Yen and Seow, 2012). Smoke outflows have cancer-causing and mutagenic properties in examines directed on *in vitro* frameworks and creature models. At the populace level, there is epidemiological proof that biomass fills are related to respiratory and cardiovascular ailments, for example, lower respiratory tract contaminations, chronic obstructive lung ailment and coronary illness (Dherani et al., 2008; Black et al., 2010). The fragmented burning of these strong energizes brings about a significant part of the fuel vitality being discharged as conceivably lethal toxins, including particles of fluctuating sizes, carbon monoxide (CO), nitrogen dioxide, unstable and semivolatile natural mixes (e.g., formaldehyde and benzo[a]pyrene), methylene chloride, and dioxins (Naeher et al., 2007). The utilization of strong energizes, basically for cooking, has been evaluated to be answerable for > 3.5 million unexpected losses for each year (in addition to an extra 0.5 million passing from open-air contamination because of family fuel use) and 110 million handicap balanced life years (DALYs) (Lim et al., 2012).

Ndindeng et al. (2019) assessed five diverse rice husk top-lit updraft (TLUD) gasifier cook-stoves for use in cooking tasks in Africa. This study showed that fan-helped cook-stoves particularly PO150 recorded better warm and discharge files and more secure to utilize contrasted with the characteristic draft gasifier stove (Mayon). Despite the fact that PO150 administrator may securely utilize the stove for cooking in all around ventilated conditions, questions despite everything exist regarding whether a few mixes transmitted by the stove can taint the nourishment being cooked and apply poisonous impacts on purchasers. Be that as it may, as far as anyone is concerned, no study on the *in vivo* toxicity of rice husk as a fuel has been depicted in the writing. In this manner, in the present examination, we planned to explore the toxicity (both oral acute and sub-chronic) of refined water bubbled on a P0150 gasifier utilizing rice husk as fuel on rodents.

MATERIALS AND METHODS

Operation of the stove and preparation of the water sample

PO150 gasifier operates on bunch mode and on a constrained draft framework which is a fan with anenergizing limit of 0.78 kg per cluster and a group enduring 30 min. At the point when the rice husk in the gasifier was totally spent, the biochar was disposed of before new fuel was placed in the gasifier to start another vitality generation process. So as to lessen the fuel topping off time and guarantee smooth cooking of dishes whose cooking time is higher than the most extreme consuming time for bunch type gasifiers, stove exchanging was utilized as recently portrayed (Ndindeng et al., 2019). Quickly, two PO150 gasifiers, A1 and A2 were delivered and utilized for the study. When A1 was running, A2 was loaded up with rice husks and set close by. The gasifier A2 was lit when the consuming time in A1 was 5 min to halting time. When A1 halted, the pot was moved to A2 and the biochar in A1 chamber disposed of, new fuel-filled and set close by.

Refined water was heated up each day during the time of the trial (28 days). The fan-helped gasifier PO150 (Figure 1) was put in a stay with a window and entryway shut.

Five liters (5 L) of refined water were placed in an open pot with 10L of limit. Since the preparing time of nourishment differs (10 min to 2 h), the time the water continues bubbling on the stove is basic, and the more it is extended, the better it will be to take into account any potential poisons from the stove emanations to break up in the water. The water was in this manner permitted to bubble for about 1h from the breaking point on the stove utilizing the rice husk as fuel. This water was then cooled to room temperature before being

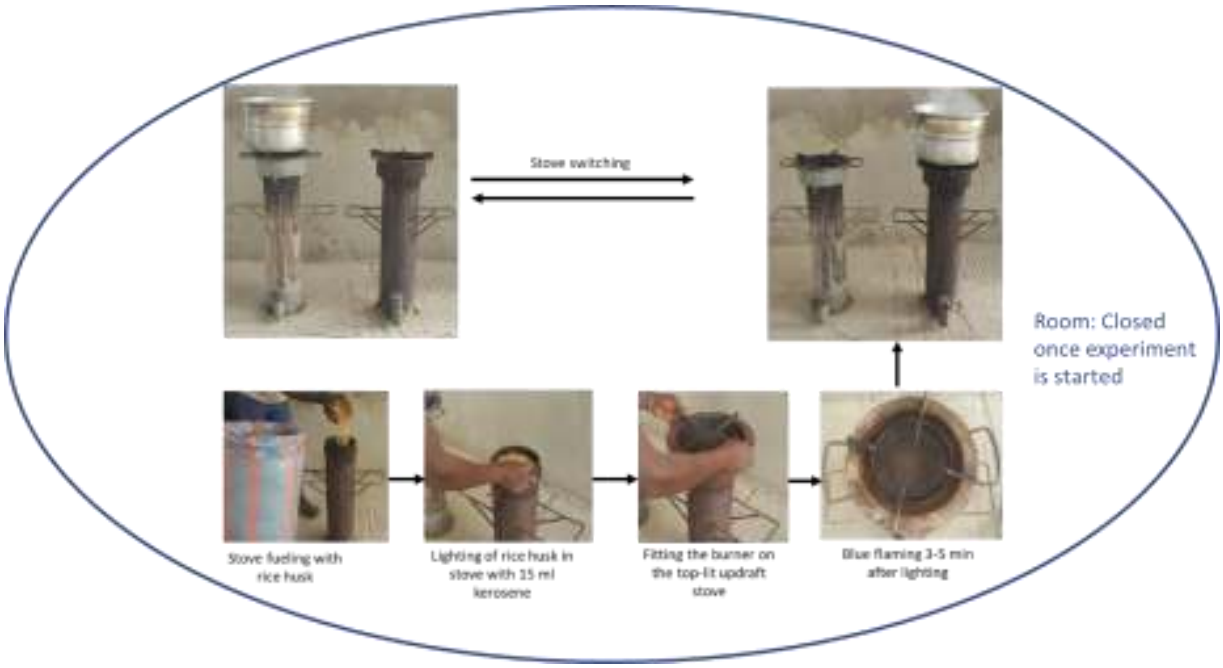

Figure 1. Schematic presentation of the setup for boiling water with a PO150 fan-assisted rice husk fuelled gasifier stove.

controlled 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 70-110 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 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 animals. Authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Reg. Nº. 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 LD_{50} 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.

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/100g bw, according to the Organization for Economic Cooperation Development (OECD) guidelines 423(OCDE, 2001). Eight (8) healthy Wistar female rats were randomly divided into 2 groups (4 females per group).On 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 unique dose of 2 ml/100g bw distilled water that was boiled with rice husk. Food was withheld for a further 4 h after giving the water. Animals were weighed every 2 days and were observed individually for general behaviour and body weight changes, toxic symptoms, and mortality during the first 30 min, periodically during the first 4 h after the administration of the unique dose of 2 ml/100 g bw boiled water, for a total of 14 days. During this period, signs of toxicity including change in coat, 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-chronictoxicity 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 1mL/100g of body weight, however in the case of aqueous solutions, 2 mL/100g body weight (bw) can be considered (OCDE, 2008). Forty (40) 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/100g), medium dose (1 ml/100g) and high dose (2 ml/100g) 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 28 days, groups 2- 4 were sacrificed while the physiological condition of the rats of group 5was restored for another 2 weeks (with food and water supplied *ad libitum*). Group 5 was orally administered samples of distilled water boiled with high dose (2 ml/100g) for 28 days but not sacrificed at the end of 28 days as Groups 1-4. Group 5 was observed for additional 14 days and sacrificed on the 42nd day. Surviving rats were anesthetized with carbon dioxide and blood samples were obtained from the eyes of the rat 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: final weight; W_i: 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 nonheparinized 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. Creatine, uric acid and total bilirubin and protein were analyzed using the method described by Timothy et al. (2015). Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) assays 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 kidney 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, cleared 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 tests to determine significant differences between values. The value of $p < 0.05$ was considered statistically significant.

RESULTS

Acute oral toxicity test

In all 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/100g 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. This result suggests 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 behavior, organ and body weight

The administration of various doses of water boiled with rice husk as fuel (0.5, 1 and 2 ml/100g 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 2B).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. Figures 3A and 3B show the relative organ

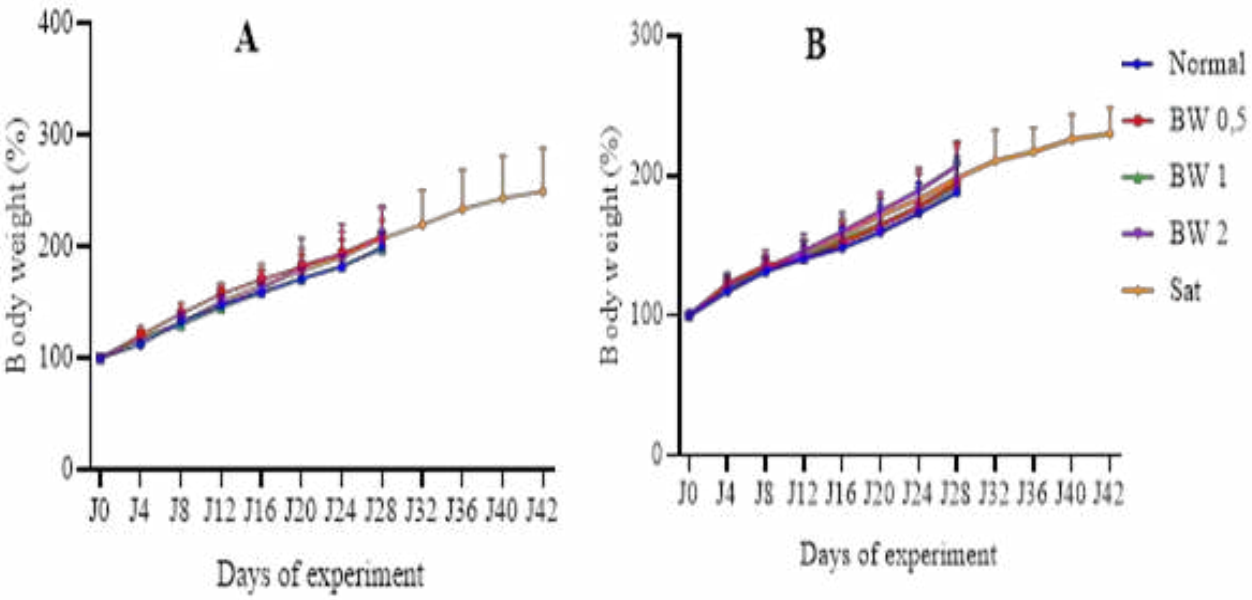

Figure 2. Effect of water boiled with rice husk on the body weight of male (A) and female (B) wistar rats. n = 4. Each value represents mean ± SD. Control: healthy rats that received distilled water; Sat: Rats that received 2 ml/100 g bw of boiled water and observed 14 days after the end of the experiment, 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.

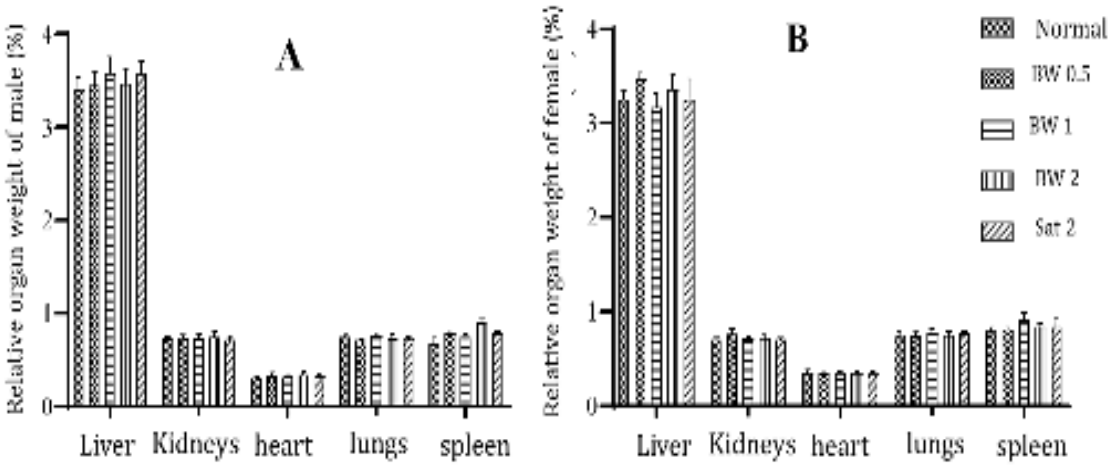

Figure 3. Relative organ weights of male (A) and female (B) rats that received different doses of the boiled water for 28 days. n = 4. Each bar represents mean ± SD. Control: Healthy rats that received distilled water; Sat: Rats that received 2 ml/100g bw of boiled water and observed 14 days after the 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.

weights of the male and female rats after 28 days of administration of the boiled water sample. The relative organ weight of the liver and kidneys, heart, lungs and spleen evaluated and calculated at necropsy in the treated groups did not show any significant difference ($p > 0.05$) from the control.

Effects on biochemical and haematological parameters

The results of the biochemical parameters are recorded on Table 1. The results for male rats showed no significant difference in some parameters (ALT, AST,

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

Parameter	Group				
	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
Bilirunin (mg/l)	0.19 ± 0.01	0.19 ± 0.03	0.23 ± 0.04	0.26 ± 0.01	0.18 ± 0.02

Table 2. Biochemical profile of female rats that received different doses of boiled water for 28 days.

Parameter	Group				
	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. Control: healthy rats given distilled water; Sat: Rats that received 2 ml/100g of boiled water and observed 14 days after the end of the experiment, BW 0.5, BW1 and BW 2 represent groups of rats that received boiled water doses at doses of 0.5, 1 and 2 ml/100 g bw respectively.

total protein, urea and total bilirubin) at all treatment doses except creatinine which significantly ($P<0.05$) increased at the dose of 1 ml/ 100g compared to the control.

In the female rats, uric acid and total bilirubin recorded significant differences, at the dose of 2 ml/100g administered. The analyses showed significant increase ($P<0.05$) in uric acid while total bilirubin significantly decreased, with more decrease14 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 (Table 2a and b). Analysis of variances showed no significant difference in most of the parameters except of platelets ($P<0.05$) compared to the control in the male rats. However, in the female rats, 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 to the control.

Histopathology study

Figures 4 and 5 showed 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 rats from the control group and those given a low dose (0.5 ml/100g bw), medium dose (1 ml/100g bw) and high dose (2 ml/100g bw), showed a normal histology.

DISCUSSION

As an initial step, an oral acute toxicity study was directed, it was seen that independent of the sex and the treatment, an expansion in weight of a similar sufficiency was seen with no measurable contrasts ($p<0.05$) between the experimental groups. Weight increment means that development. The sensible homogeneous expanding pattern of body and organ weight in all the rodent's gatherings can be taken as a sign of the low effect of various medicines on creature taking care of and wellbeing. Njayou et al. (2010) saw that body weight may increment or decline with connection to sex, non-attendance of toxicity or instigated anorexia. The

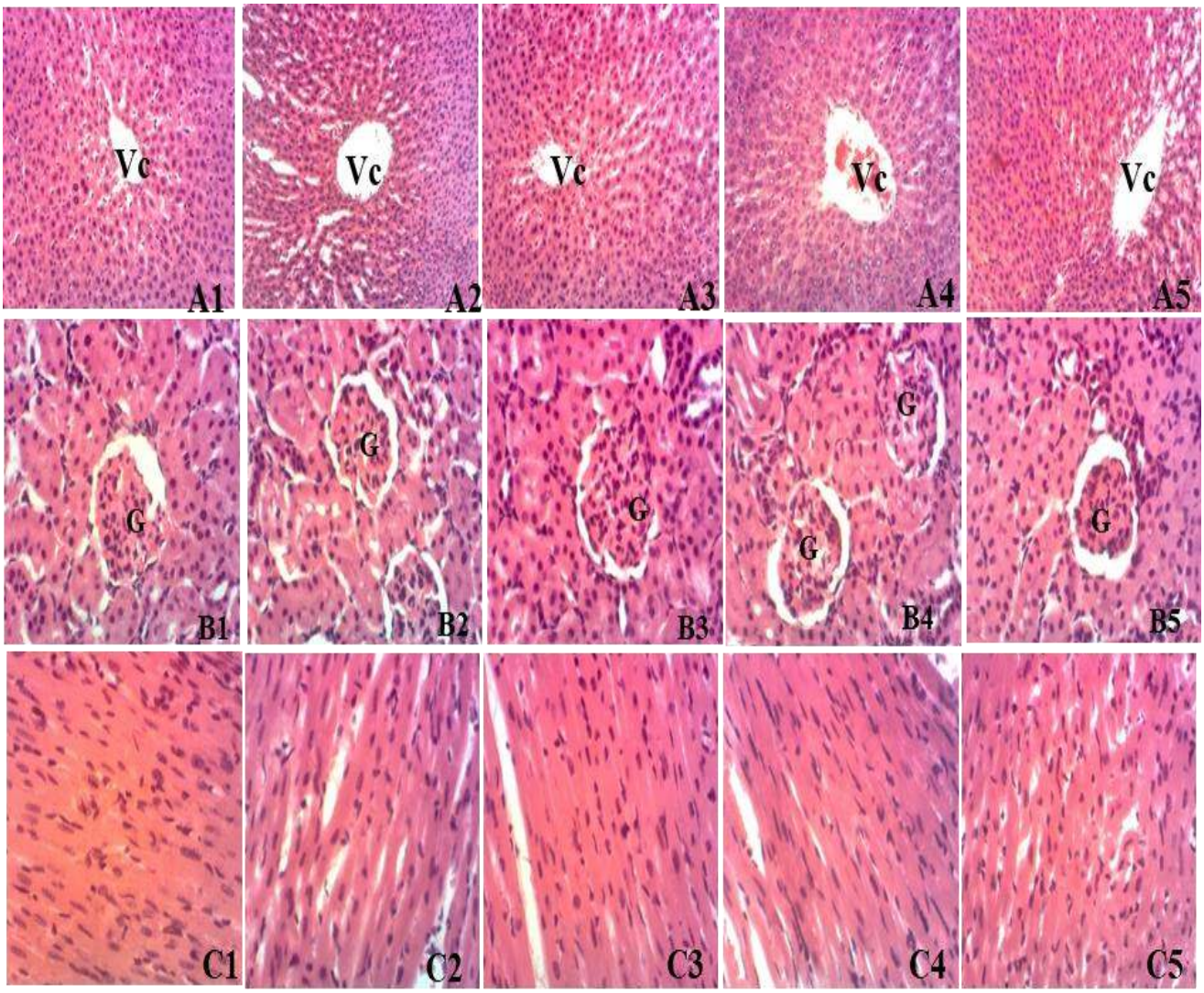

Figure 4. Histopathological examination of organs of male rats in a sub'chronic oraltoxicity study. (A–C) the liver, kidney, and heart respectively; and (1–5) the control, the low, middle, high dose and the satellite (highest) dose groups, respectively. G, Glomerulus; CV, Central Vein.

outcomes show that there was no mortality or irregular conduct or indications of toxicity after the organization of the most noteworthy portion (2 ml/100 g BW) for as long as 14 days. This shows, as indicated by the naming and grouping of acute foundational toxicity prescribed by the OECD, the deadly portion might be over this portion limit, known as Class 5 status (OECD, 2001). Further examination was directed to assess the sub-chronic toxicity of rice husk bubbled water for 28 days in rodents.

Substances directed in chronic sickness conditions may require a toxicological assessment of rehashed dosages (sub-chronic toxicity study), since everyday use may bring about aggregation in the body with slow consequences for tissues and organs (Abotsi et al., 2011; Bariweni et al., 2018). Twenty-eight (28) days of oral toxicity study of water overflowed with rice husks, at portions of 0.5, 1 and 2 ml/100 g BW, did not give any antagonistic clinical indications or negative effects on conduct and mortality in the experimental groups. Changes in feed and water admission and body weight gain have been utilized as a marker of the general wellbeing status of exploratory creatures (El Hilaly et al., 2004). Feed utilization is directed through a few complex natural instruments that can guarantee generally consistent body weight over extensive stretches of time (Kuriyan et al., 2007). No critical distinction in body weight addition of the male and female rodents contrasted with the control was recorded. Once more, in toxicity contemplates, changes in the heaviness of organs are delicate markers of toxicity, consequences for compounds, physiologic unsettling influences and target organ injury (Michael et al., 2007). An expansion in organ weight recommends the event of hypertrophy while an abatement proposes corruption in the objective organ

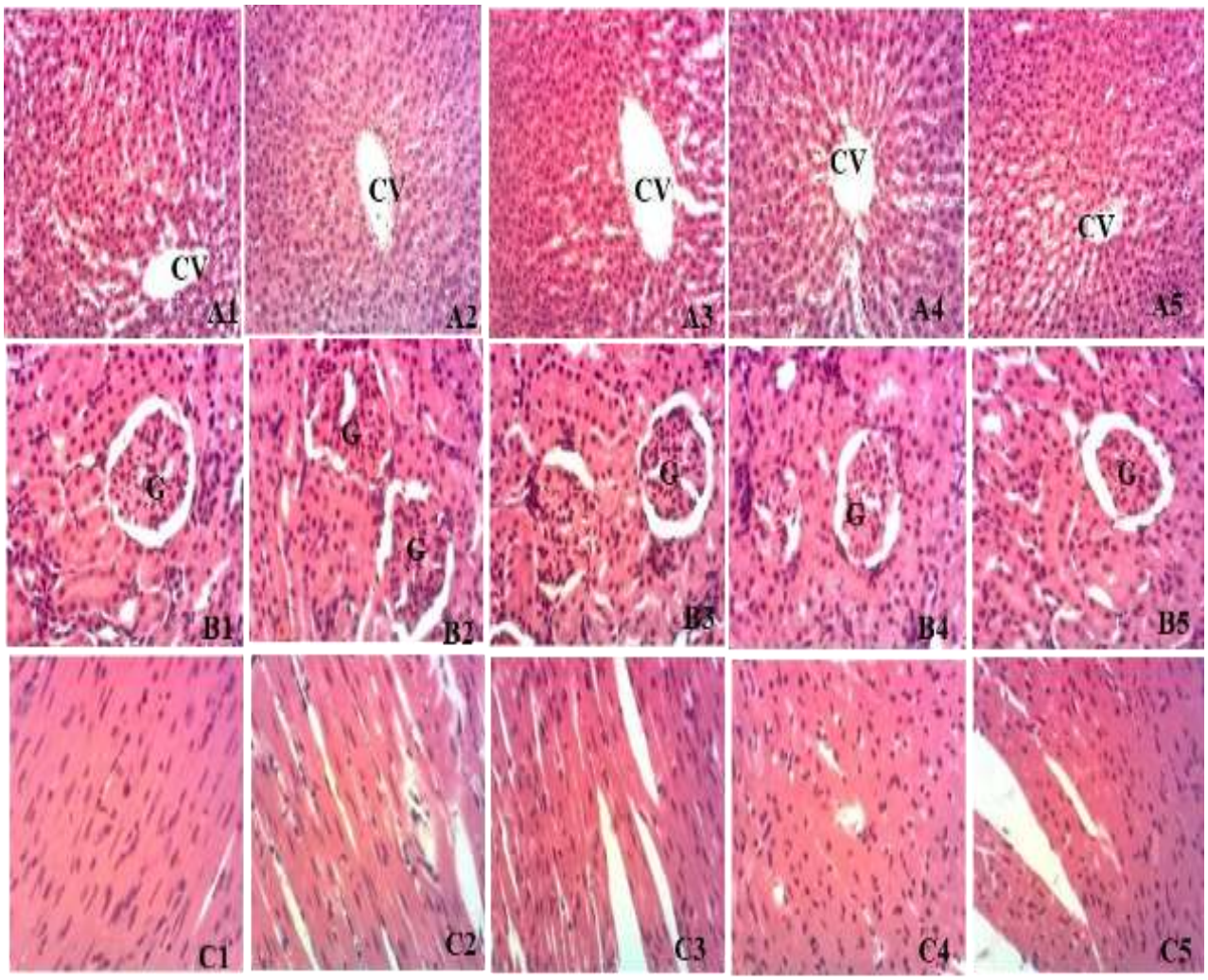

Figure 5. Histopathological examination of organs of female rats in a sub-chronic oral toxicity study: (A–C) the liver, kidney, and heart respectively; and (1–5) the control, the low, middle high dose and the satellite (highest) dose groups, respectively. G, Glomerulus; CV, Central Vein

(Teo et al., 2002). Right now, relative organ weight of the liver and kidneys, heart, lungs, and spleen assessed in the experimental groups did not show a critical contrast in both genders at all dosages contrasted with the Control.

The study of biochemical parameters are pointers of toxicity, raising the adequacy of the establishment of toxicity on imperative organs. Right now, as creatinine in male, all-out bilirubin, and uric corrosive in female rodents indicated critical contrasts between the experimental groups and controls. The portion of 1 ml/100g in guys altogether expanded creatinine and at 2 ml/100g bw directed in females, uric corrosive was additionally essentially expanded ($P<0.05$) yet all-out bilirubin fundamentally diminished. Creatinine is a discharge result of muscle movement, which circles in the blood. Its end is solely renal, so there is a relationship between's creatinine levels and renal capacity. Most creatinine that is disposed of by the kidneys is unreservedly separated in renal glomeruli, and a little part is sifted by the rounded segment, which is a decent pointer of renal-glomerular capacity (Bohinski, 1991; Raju et al., 2016; Ghorbel et al., 2016; Belhadj et al., 2018). The lessening of these parameters would show the hepatoprotective activity of the bubbled water test at this portion. Uric corrosive is the final result of nucleic corrosive digestion (Wallace et al., 2004). It is shaped by the liver and chiefly discharged by the kidneys (65-75%) and digestive organs (25-35%) (Álvarez and Macarron., 2010). In the present study, blood uric corrosive was high in female rodents (2 ml/100g), proposing kidney harm (Raju et al., 2015, 2016; Belhadj et al., 2018). The working of the liver was evaluated by the serum all-out protein, bilirubin and egg whites. Expansion in these parameters is generally observed in destructive conditions or following a high protein diet (Tietz et al., 1994). Our study demonstrated a noteworthy decline in

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	426.3±3,2*	368.0±5,5

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. Control : healthy rats given distilled water; Sat: Rats that received 2 ml/100 g of boiled water and observed 14 days 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, 1 and 2 ml/100 g bw respectively.

complete bilirubin in the female rodents at the portion of 2 ml/100g recommending the lethal impact of the bubbled water on the liver of the creatures. The complete protein serum level did not contrast altogether from the benchmark group. This shows the impact on the liver could be a gentle harmful impact influencing just the female rodents. For the most part, it created the impression that the water influenced the female at a lower portion rather than the guys, which were not influenced.

Hematopoiesis is the procedure of platelet arrangement. Analysis of the hematological parameters is significant in evaluating the poisonous impacts of test substances, just as in deciding the physiological and obsessive status of the body, as varieties in these parameters may show toxicity related with the test substances and different illnesses and conditions, including frailty, leukemia, responses to aggravation and diseases (Olson et al., 2000; Martini et al., 2012). There was no noteworthy contrast in a few parameters, for example, red platelet checks, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin focus, white platelet tallies, lymphocytes, and platelet between the treated gatherings and the benchmark group, demonstrating that the bubbled water had no impact on the circling platelets of the tried creatures (Tables 3 and 4). For monocytes, the examinations indicated a huge increment at the portion of 2 ml/100 g, the number of granulocytes likewise essentially expanded ($P<0.05$) at all the dosages when contrasted with the control in females. Notwithstanding, these distinctions acquired at the moment did not show a hematological change since they are inside the ordinary scope of these parameters for good wellbeing (Giknis and Clifford, 2008).

These distinctions got between the tried creatures and

the control could be clarified by the nearness of suspended issues; for example, the rice husk debris (RHA) right now. This RHA is found in the bubbled water through the ventilation produced by the fan. As indicated by Xu et al. (2012), debris has the most elevated extent of silica content among all plant buildups. The normal organization of very much consumed RHA is 90% undefined silica. Our discoveries are in accordance with those of Wai et al. (2017) who explored the *in vivo* toxicity of Silica nanoparticles (SiNPs) of 150 nm in different measurements by means of intravenous organization in mice and demonstrated that SiNPs were biocompatible and ok for *in vivo* use in mice.

The histology of the kidneys, liver, and heart in the male and female rodents did not create any lethal changes, in spite of introducing a few changes in biochemical tests, the histological study proposes the wellbeing of the rice husk bubbled water in these organs. This shows rice husk utilized as a fuel in PO150 gasifier stove is non-harmful 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 gbw) 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 in long term.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors gratefully appreciate the Laboratory of Animal Physiology, Department of Animal Biology, University of Yaoundé I-Cameroon for providing the facilities to carrying out this research work.

REFERENCES

Abotsi WK., Ainooson GK, Gyasi EB, Abotsi WKM (2011). Acute and subacute toxicity studies of the ethanolic extract of the aerial parts of Hilleria latifolia (Lam.) H. Walt. (Phytolaccaceae) in rodents. West African Journal of Pharmacy 22:27-35.

Anderson PS, Wendelbo P, Reed TB, Belonio AT (2008). Super-clean combustion of solid biomass fuels in affordable TLUD cook stove. Beyond Firewood: Exploring alternative fuels and energy technologies.

Alvarez-Lario B, Macarron-Vicente J (2010). Uric acid and evolution. Rheumatology (Oxford) 49:2010-2015.

Baravalle C, Salvetti NR, Mira GA, Pezzone N, Ortega HH (2006). Microscopic characterization of follicular structures in letrozole-induced polycystic ovarian syndrome in the rat. Archives of Medical Research 37(7):830-839.

Bariweni MW, Yibala OI, Ozolua RI (2018). Toxicological studies on the aqueous leaf extract of Pavetta crassipes (K. Schum) in rodents. Journal of Pharmacy Pharmacognosy Research 6(1):1-16.

Belhadj BA., Dilmi BA., Mezaini A, Belhadri A, Benali M (2018). Effect of oral exposure to acrylamide on biochemical and hematologic parameters in Wistar rats. Drug and Chemical Toxicology 42(2):157-166.

Bhupinder Singh (2018), in Waste and Supplementary Cementitious Materials in Concrete,. https://www.sciencedirect.com/topics/engineering/rice-husk-ash.(Provide valid link)

Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, Jha P, Campbell H, Walker CF, Cibulskis R, Eisele T, Liu L, Mathers C (2010). Global, regional, and national causes of child mortality in: a systematic analysis. Lancet 375(9730):1969–1987.

Bohinski C (1991). 5th ed. Editorial Addison – Wesley Iberoamericana; Wilmington: Bioquímica.

Brown JR, Thornton JL (1957). Percivall Pott (1714-1788) and chimney sweepers' cancer of the scrotum. British Journal of Industrial Medicine 14:68-70.

Dherani M, Pope D, Mascarenhas M, Smith KR, Weber M, Bruce N (2008). Indoor air pollution from unprocessed solid fuel use and pneumonia risk in children aged under five years: a systematic review and meta-analysis. Bulletin of the World Health Organization 86(5):390-398.

El Hilaly J, Israili ZH, Lyoussi B (2004). Acute and chronic toxicological studies of Ajuga iva in experimental animals. Journal of Ethnopharmacology 91(1):43-50.

GEMS: Global Environment Monitoring System (1990). Assessment of Urban Air Quality. United Nations Environment Programme/World Health Organization, 1998. (Unpublished document cited in Chen BH, Hong CJ, Pandey MR, Smith KR. Indoor air pollution in developing countries. World Health Stat Q 43(3):127p.

Ghorbel I, Maktouf S, Fendri N, Jamoussi K, Ellouze Chaabouni S, Boudawara T, Zeghal N (2016). Co-exposure to aluminum and acrylamide disturbs expression of metallothionein, roinflammatory cytokines and induces genotoxicity: biochemical and histopathological changes in the kidney of adult rats. Environmental Toxicology 9:1044-1058.

Giknis MLA, Clifford CB (2008). Clinical laboratory parameters for Crl: CD (SD) rats Charles River Laboratories.

IRRI. World Rice Statistics Online Query Facility (2019). Results generated on Dec 29, 2019 19:01. Los Baños: International Rice Research Institute; http://ricestat.irri.org:8080/wrsv3/entrypoint.htm

Kuriyan R, Raj T, Srinivas SK, Vaz M, Rajendran R, Kurpad AV (2007). Effect of Caralluma fimbriata extract on appetite, food intake and anthropometry in adult Indian men and women. Appetite 48(3):338-344.

Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K., Adair-Rohani H, et al., (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2224-2260.

Martini FH, Nath JL, Bartholomew EF (2012). Fundamental of Anatomy and Physiology, 9th ed.; Pearson: San Fransisco, CA, USA, pp. 642–645, 776.

Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, Johnson JK, Schafer K, (2007). Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicologic Pathology 35(5):742-750.

Naeher LP, Brauer M, Lipsett M, Zelikoff JT, Simpson CD, Koenig JQ, Smith KR (2007). Woodsmoke health effects: a review. Inhalation Toxicology 19(1):67-106.

Ndindeng SA, Mbassi JEG, Mbacham WF, Manful J, Graham-Acquaah S, Moreira J, Dossou J, Futakuchi K(2015). Quality optimization in briquettes made fromrice milling by-products. Energy for Sustainable Development 29:24-31.

Ndindeng SA, Marco W, Sidi S, Koichi F (2019). Evaluation of fan-

assisted rice husk fuelled gasifier cookstoves for application in sub-Sahara Africa. Renewable Energy 925-937.
Njayou FN, Moundipa PF, Donfack JH, Djamen Chuisseu JH (2010). Hepato-protective, antioxidant activities and acute toxicity of a stem bark extract of Erythrina senegalensis DC. International Journal of Biological and Chemical Sciences 4(3):738-747.
OECD: Organisation for Economic Co-operation and Development, (2001).Guidelines for The Testing of Chemicals: Acute Oral Toxicity—Fixed Dose Procedure, OECD/OCDE 423. Adopted: 17th December 2001.
OECD: Organisation for Economic Co-operation and Development, (2008). Guidelines for The Testing of Chemicals: Repeated Dose 28-Day Oral Toxicity Study in Rodents, Updated with Parameters for Endocrine Effects, Guideline 407, Head of Publications Service, Paris, France.
Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W,Dorato M, Van Deun K, Smith P, Berger B, Heller A (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. Regulatory Toxicology and Pharmacology 32:56-67.
Raju J, Kocmarek A, Roberts J, Taylor M, Patry D, Chomyshyn E, Caldwell D, Cooke G, Mehta R, (2016). Lack of adverse health effects following 30-weeks of dietary exposure to acrylamide at low doses in male F344 rats. Toxicology Reports 3:673-678.
Raju J, Roberts J, Taylor M, Patry D, Chomyshyn E, Caldwell D, Cooke G, Mehta R (2015). Toxicological effects of short-term dietary acrylamide exposure in male F344 rats. Environmental Toxicology and Pharmacology 39:85–92.
Reitman S, Frankel S (1957). A colorimetric method for the determination of serumglutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 28(1):56-63.
Rodier J, Mallein R (1983). Enzymologie. Transaminases sériques (S.G.O.T et S.G.P.T). In. Manuel de Biochimie Pratique, Maloine SA (ed). Paris. pp. 396-400.
Smith KR, Balakrishnan K, Butler C, Chafe Z, Fairlie I, Kinney P, Kjellstrom T, Mauzerall DL, McKone TE, McMichael AJ, Schneider M (2012). In: Global Energy Assessment: Toward a Sustainable Future, (Johansson TB, Patwardhan A, Nakicenovic N, Gomez-Echeverri L, eds). New York: Cambridge University Press for International Institute for Applied Systems Analysis, pp. 255-324;. Energy and health.
Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V, (2002). A 90-day oral gavage toxicity study of d-methylphenidate and d, l-methylphenidate in Spraguee Dawley rats. Toxicology 179(3):183-196.
Tietz WN, Prude EL, Sirgard-Anderson O (1994). Tietz Textbook of Clinical Chemistry. WB Saunders London, UK.
Timothy SY, Helga BI, Bomai HI, Musa AH (2015). Acute and sub-chronic toxicity study of the aqueous and ethanolic extracts ofMitragyna inermis bark in albino rats. International Journal of Pharmacology and Toxicology 5(1):24-32.
Torres-Duque C, Maldonado D, Pérez-Padilla R, Ezzati M, Viegi G (2008). Biomass fuels and respiratory diseases. A review of the evidence. Proceedings of the. American Thoracic Society 5:577–590.
Wai TC, Cheng CL, Jen S, Shang TT, Chih KL, Mei LC, Hung CL, Chun YY, Shao YH (2017). In vivo toxicologic study of larger silica nanoparticles in mice. International Journal of Nanomedicine 12:3421-3432.
Wallace KL, Riedel AA, Joseph-Ridge N, Wortmann R (2004). Increasing prevalence of gout and hyperuricemia over 10 years among older adults in a managed care population. Journal of Rheumatology 31:1582–1587.
Wei-Yen L. and Seow A., (2012). Invited review series: air pollution and lung health: Biomass fuels and lung cancer. Respirology 17:20-31.
Xu W, Lo TY, Memon SA (2012). Microstructure and reactivity of rich husk ash. Construction and Building Materials 29:541-547.