



## Toxicity, mutagenicity and trace metal constituent of *Termitomyces schimperi* (Pat.) R. Heim (Lyophyllaceae) and kaolin, a recipe used traditionally in cancer management in Cote d'Ivoire

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### ARTICLE INFO

#### Keywords:

Termitomyces schimperi  
Kaolin  
Acute/sub-chronic toxicity  
Mutagenicity  
Trace metals

### ABSTRACT

**Ethnopharmacological relevance:** Some local communities in Cote d'Ivoire use the mushroom *Termitomyces schimperi* combined with kaolin (TSK) to manage various cancers in patients. However, there is a paucity of data on toxicity, mutagenicity and trace metal constituent of TSK.

**Aim of the study:** We sought to investigate the acute and sub-chronic toxicities, mutagenic potential, and trace metal constituents of TSK.

**Materials and methods:** To assess acute toxicity, single doses (1000, 3000 and 5000 mg/kg) of aqueous extract of TSK were administered *per os* to Sprague Dawley (SD) rats on Day 1. The rats were then monitored for 13 consecutive days. Sub-chronic toxicity was evaluated by daily administration of 200 and 500 mg/kg of the extract *per os* for 90 consecutive days. SD rats used as control received distilled water. Signs of toxicity, changes in body weight and mortality were monitored. After the aforementioned monitoring processes, rats were sacrificed and blood collected for full blood count and biochemistry analysis. Animal organs were also collected for histopathological examination. The mutagenic potential of the aqueous extract of TSK (10000 µg/mL) on TA98 *Salmonella typhimurium* was estimated. Additionally, energy-dispersive X-ray fluorescence (ED-XRF) method was employed to determine trace metal constituents of TSK.

**Results:** Single-dose administration of 5000 mg/kg of TSK did not cause any death in the SD rats; thus, LD<sub>50</sub> was above 5000 mg/kg. Administration of 1000 and 3000 mg/kg of the aqueous extract of TSK did not cause any significant change in behaviour and body weight of SD rats during the 14-day monitoring period. However, the mean corpuscular volume and the mean corpuscular haemoglobin concentration increased significantly ( $p < 0.01$ ) among rats administered 1000 and 3000 mg/kg of TSK. There was a significant increase ( $p < 0.0001$ ) in alanine transaminase levels in rats administered 1000 and 3000 mg/kg of TSK extract compared with control. Conversely, there was a significant decrease ( $p = 0.0122$ ) in serum creatine level among rats administered 1000 and 3000 mg/kg of TSK extract compared with control. After 14 days, there were minimal changes with isolated organs of TSK-treated and control rats. Furthermore, 90-day treatment with extract of TSK caused no significant

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<https://doi.org/10.1016/j.jep.2021.114147>

Received 19 February 2021; Received in revised form 6 April 2021; Accepted 23 April 2021

Available online 28 April 2021

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change in parameters assessed. TSK induced frameshift gene mutation in *S. typhimurium* before ( $p < 0.05$ ) and after metabolic activation ( $p < 0.001$ ). Elemental analysis of TSK revealed the presence of toxic (aluminium) or potentially toxic (silver, radium, titanium and zirconium) elements.

**Conclusions:** The aqueous extract of TSK showed no toxicity (acute and sub-chronic) at doses tested. These findings are consistent with the absence of heavy metals (i.e., cadmium) and potentially toxic elements (i.e.,

### Abbreviations

ED-XRF	Energy dispersive x-ray
HPLC	High-performance liquid chromatography
LD <sub>50</sub>	Lethal dose 50
NOAEL	No-observed adverse effects level
NPFAC	National programme for fight against cancer
NPTM	National programme for promotion of traditional medicine
OECD	Organisation for economic co-operation and development
SD	<i>Sprague Dawley</i>
TS	<i>Termitomyces schimperi</i>
TSK	<i>Termitomyces schimperi</i> and Kaolin

uranium) in TSK samples analysed. TSK showed some level of mutagenic potential. Further mutagenic and chronic toxicity studies on TSK are required.

## 1. Introduction

The medicinal, ecological, and nutritional properties of mushrooms, as well as poisonous potential of some species are well documented (Bernart, 2005; De Castro et al., 2016; Govorushko et al., 2019; Lindquist et al., 2005). Mushrooms such as *Termitomyces schimperi*, *Lentinula edodes* and *Ganoderma lucidum* have been used traditionally to treat hepatitis, asthma, hypertension, diabetes and cancer (Miles and Chang, 2004). Others such as *Agaricus moelleri*, *Amanita phalloides* and *Rubroboletus satanas* are known to be poisonous to humans (Gopinath et al., 2011; Govorushko et al., 2019). Clinical manifestations of poisoning from mushrooms could range from mild gastrointestinal disturbances to severe organ injury (Gopinath et al., 2011; Govorushko et al., 2019).

Reports suggest that heavy metals from polluted environments can easily be absorbed through the mycelium of mushrooms and accumulate in the fruiting body (Melgar et al., 2016; Türkmen and Budur, 2018). Accumulation of heavy metals may increase the mutagenic potential of mushrooms (Falade et al., 2008). In humans, toxicity from heavy metal-contaminated mushrooms is less severe than one caused by poisonous mushrooms (Khani et al., 2017; Liu et al., 2015; Melgar et al., 2016; Nharingo et al., 2015).

*Termitomyces* Heim is a genus of mushrooms belonging to the family Lyophyllaceae. *Termitomyces* grows symbiotically on termite nests. This association is essential for the survival of both mushrooms and termites. This makes *ex-situ* cultivation of *Termitomyces* mushrooms often challenging. There are about 20 known *Termitomyces* species in Africa and Asia (Koné et al., 2018; Kuja et al., 2014). Some examples are *T. clypeatus*, *T. letestui*, *T. robustus* and *T. schimperi*. These *Termitomyces* species have a wide range of medicinal properties, some of which include antioxidant, antimicrobial, antiulcer, immunostimulatory and antitumor (Hsieh and Ju, 2018; Mahamat et al., 2018; Nyegue et al., 2003). Reports show that *T. schimperi* is used in the management of kwashiorkor (protein malnutrition), hypertension, obesity and rheumatism (Obodai et al., 2014; Patel and Goyal, 2012).

A survey by our team, in collaboration with the Ivorian National Programme for Promotion of Traditional Medicine (NPPTM) and the

National Programme for Fight against Cancer (NPFAC), revealed that *T. schimperi* is used in combination with kaolin to manage breast cancer in Cote d'Ivoire (unpublished data). Kaolin is a common mineral of hydrous aluminium silicate. Traditionally, kaolin is used to manage diarrhoea, ulcers and inflammation of the large intestine (Williams and Hillier, 2014). However, some reports have suggested that long-term exposure to kaolin causes specific pneumoconiosis known as kaolinosis (Finkelman, 2019). High concentrations of kaolin in circulation have been shown to cause mild toxicity on sensitive endothelium cells (Bowman et al., 2011).

It is noteworthy that the mutagenic properties of some agents are employed clinically in chemotherapy to inhibit cancer cell proliferation (Ferguson and Pearson, 1996; Parrella et al., 2015). There is, however, paucity of data on toxicity and mutagenicity of *T. schimperi* and kaolin. The current study, therefore, sought to assess acute and sub-chronic toxicities, mutagenic potential, and trace metal constituent of *T. schimperi* combined with kaolin (TSK).

## 2. Materials and methods

### 2.1. Mushroom collection and extraction

The fruiting bodies of *T. schimperi* were collected from the wild in Agboville (Latitude: 5° 55' 40.84" N; Longitude: 4° 12' 47.48" W), Cote d'Ivoire, in September 2013. No permission was required to collect these mushrooms. The mushrooms were identified by Mrs Adjoa Agnes Koffi (a herbalist) and authenticated by the Late Professor Laurent Ake-Assi of the National Centre of Floristic (Felix Houphouët-Boigny University, Abidjan-Cocody, Cote d'Ivoire). A voucher specimen of the mushroom (N° 13098) was deposited at the herbarium of this centre. Kaolin powder was purchased from the market at Abidjan-Cocody, Cote d'Ivoire, and authenticated at the Department of Geology (Felix Houphouët-Boigny University, Abidjan-Cocody, Cote d'Ivoire). Preparation of the mixture of TSK and subsequent extraction were as done traditionally. The fruiting bodies of *T. schimperi* were washed with distilled water, air-dried for 2 weeks, ground into a powder, and mixed with kaolin in a 40:60 ratio. Three litres of water was added to 300 g of the mixture and

left overnight (at 4 °C). This mixture was then dried in an oven at 55 °C. The dried material was ground into a fine powder. Afterwards, 250 g of the crude powder was dissolved in 2.5 L of distilled water, stirred for 4 h using a magnetic stirrer and extracted by cold maceration for 24 h. Supernatant was filtered using gauze pads and Whatman No. 1 filter paper. The remaining residue was re-extracted till exhaustion and subsequently filtered. Filtrates were combined and taken through freeze-drying (Heto Powerdry LL3000 Freeze Dryer, USA) to obtain 10.38 g of powdered extract, giving a percentage yield of 3.46% w/w.

## 2.2. High-performance liquid chromatography (HPLC) fingerprint of *T. schimperii*

HPLC fingerprint analyses of *T. schimperii* was carried out as previously described (Molehin et al., 2014), with slight modifications. Briefly, an Agilent 1100 system (Santa Clara, CA, USA), with a quaternary pump, autosampler, diode array detector (DAD), and HP ChemStation Software, was used. Chromatographic separation was performed on a Tskgel ODS C18 (250 × 4.6 mm i. d., 5 µm particle size) analytical column maintained at 40 °C. The mobile phase was made up of water in 0.1% Phosphoric acid (A) and methanol (B). The flow rate of mobile phase was 1 mL/min. The gradient programme was set as follows: 0–5 min, 5–10 %B; 5–10 min, 10–20 %B; 10–12 min, 20–50 %B; 12–15 min, 50–80 %B; 15–20 min, 80–80 %B; 20–23 min, 80–5%B, 23–25 min, 5–5%B. The injection volume was 10 µL (10 mg/mL of samples were dissolved in 50% methanol, vortexed, sonicated for 10 min and centrifuged at 12,000 rpm for 10 min) and wavelength monitored was at 270 nm.

## 2.3. Macro- and micro-element screening with energy-dispersive x-ray fluorescence spectroscopic (ED-XRF)

Macro- and micro-elements in *T. schimperii* and kaolin mixture were estimated using a portable Olympus Vanta XRF Analyser, VMR series (Olympus, USA), according to manufacturer's protocol. The analyser operates using the energy dispersion principle. The equipment was standardised using a blank pellet made of silica. Samples (4 g) and a binder (Fluxana H Elektronik BM-0002-1, Licowax C micro powder PM-Hoechstwax, Germany) were homogenized using the RETSCH Mixer Mill (MM301, Germany) and pressed with a manual hydraulic press (SPECAC, UK) to obtain pellets of 32 mm in diameter and 3 mm in thickness. Samples were measured in triplicate.

## 2.4. Animal care and safety

Protocol for this research was reviewed and approved by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana (Protocol Identification Number: CHS-Et/M.6-P1.5/2017–2018). Experiments were conducted according to internationally accepted principles for laboratory animal use and care (National Research Council Committee for the Update of the Guide for the and Use of Laboratory, 2011).

Forty-three pathogen-free male *Sprague-Dawley* (Hsd: SD strain) rats, 6–8 weeks old, weighing 180–200 g, were purchased from Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana. Animals were housed in stainless steel cages of 2 cubic feet (61 cm × 31 cm x 31 cm) with softwood shavings as bedding. The SD rats were maintained under standard laboratory conditions (temperature ~25 °C, relative humidity 60–70%, and 12 h light-dark cycle), fed with standard pellet diet (Agrimat, Kumasi, Ghana) and allowed access to water *ad libitum*. Animals were acclimatised under these conditions for 7 days before experimentation. To prevent contamination, feed and water troughs of animals were washed frequently.

## 2.5. Acute toxicity study (14 days)

Assessment of acute toxicity of the aqueous extract of TSK after oral administration was determined by the fixed-dose and lethal dose fifty (LD<sub>50</sub>) methods. The aforementioned methods were conducted according to the Organisation for economic co-operation and development (OECD) Guideline 423 (In, 2001) and as previously reported (Asiedu-Gyekye et al., 2014), with slight modifications. Briefly, SD rats were put into 4 groups of 5. Three groups (Groups 1 to 3) were administered a single dose of the TSK extract (1000, 3000 and 5000 mg/kg, respectively) via the oral route with a gavage syringe. The last group (group 4) received distilled water (control). The rats were observed hourly during the first 48 h, and once daily thereafter for 12 days. During the period, changes in mobility, salivation, sleep, lethargy, piloerection, and tremor were assessed (fixed-dose method). Also, the body weight of rats was recorded on days 1, 7 and 14. The dose that caused death in 50% of the rat population per group was deemed LD<sub>50</sub> (lethal dose test). On day 14, rats were sacrificed by cervical dislocation, and blood samples were collected. Haematology and biochemical analyses were carried out on blood using automated analysers (detailed methods subsequently described). Vital organs (lungs, liver, heart, kidneys, and spleen) were harvested from SD rats in each group and weighed. Macroscopic and microscopic histological examinations were carried out on the organs to identify any pathophysiological changes.

## 2.6. Sub-chronic toxicity study (90 days)

Sub-chronic toxicity of the TSK extract was estimated according to OCDE guidelines (OCDE, 2018), and as previously reported (Amoateng et al., 2016), with slight modifications. Briefly, 18 male SD rats, aged 6–8 weeks and weighing 180–200 g, were randomly assigned to 3 groups (6 rats/group). Aqueous extracts of TSK (200 and 500 mg/kg) were administered to first two groups via the oral route with a gavage syringe. The third group, administered distilled water, served as control. Administration was for 90 days. Animals were observed daily for clinical signs of toxicity and/or mortality. Body weight of animals was taken every week throughout the study. All rats were sacrificed at the end of the 90 days by cervical dislocation and blood samples collected for haematological and biochemistry analyses. The lungs, liver, heart, kidneys, testes and spleen were harvested and weighed. Macroscopic and microscopic histopathology examinations were performed on all organs.

## 2.7. Full blood count

Complete blood count was performed using an automated haematology analyser (URIT-5250Vet, China), according to manufacturer's protocol, and as previously reported (Asiedu-Gyekye et al., 2016). Briefly, 2 mL of blood from euthanised SD rats were drawn by cardiac puncture and then transferred into ethylenediaminetetraacetic acid (EDTA) tubes. Haematological indices determined included red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), lymphocyte count (LYM), red cell distribution width (RDW), platelet distribution width (PDW), mean platelet volume (MPV), and granulocyte (GRA).

## 2.8. Biochemistry analysis

Biochemistry analysis was performed using a biochemistry analyser (URIT-8021AVet, China), according to manufacturer's protocol, and as previously reported (Asiedu-Gyekye et al., 2016). Briefly, 1 mL of blood from sacrificed rats was collected by cardiac puncture into gel tubes. Blood was allowed to stand for 45 min at room temperature before centrifugation at 5000 rpm for 15 min. Serum obtained was pipetted and placed in cryotubes. Markers of renal function (urea and serum

creatine), liver function (albumin, total protein, globulin, total bilirubin, direct bilirubin, indirect bilirubin, aspartate aminotransferase: AST, alanine aminotransferase: ALT and alkaline phosphatase: ALP) and lipid profile (total cholesterol: TC, triglycerides: TG, high-density lipoprotein: HDL, and low-density lipoprotein: LDL) were estimated.

## 2.9. Histopathology

Histopathological analyses were performed as previously described (Asiedu-Gyekye et al., 2016). The harvested organs (lungs, liver, heart, kidneys, testes and spleen) were placed in ascending grades of alcohol (70%, 80%, 90% and absolute), thrice through xylene, infiltrated and embedded in paraffin wax. Five-micron sections were prepared and stained with haematoxylin and eosin. Slides were put in 2 xylene changes for 15 min each, and then through 3 changes of absolute ethanol for 5 min each. This was followed by taking slides through 70% ethanol for 15 min, and 50% ethanol for 15 min. The slides were then passed through tap water for 30 min, distilled water for 2 min, and then stained with Mayer's haematoxylin for 15 min. After that, the slides were passed through tap water for 30 min, eosin solution for 5 min, and 80% ethanol for 5 min. Again, slides were passed through 2 changes of absolute ethanol for 5 min, 3 changes of absolute ethanol for 5 min, and finally mounted in dibutylphthalate polystyrene xylene (DPX) mountant. Slides were examined under a light microscope (Olympus UIS 2, Japan) at magnification x40 by two independent certified histologists.

## 2.10. Mutagenic assay (Ames test)

The mutagenic potential of the aqueous extract of TSK was determined using the Muta-ChromoPlate™ two strain kit (Environmental Bio-Detection Products Inc, Ontario, Canada) which works by the principle of reverse mutation or back mutation (Ames et al., 1975). The assay was conducted according to manufacturer's protocol, and as previously described (Oppong Bekoe et al., 2020). Briefly, a bacterial strain (*Salmonella typhimurium* TA98) was grown overnight for 14 h at 37 °C in a nutrient medium. The reaction mixture, phosphate buffer and distilled water were placed in Falcon tubes. The extract of TSK or positive control (nitrofluorene) was added to separate tubes containing the reaction mixture. For determination of mutagenicity, 10000 µg/mL of the extract of TSK was used. Bacteria were added to each tube, except for the blank. The mixture was vortexed, poured into sterile reagent boats and dispensed into each well of a sterile 96-well plate. The plates were incubated for 5 days, after which all the wells showed purple colour (each well was considered a colony). The experiments were repeated with 4% S9 (rat liver enzyme) in addition to the reaction mixture. Mutagenicity was detected in the 96-well plates when there was a change in colour from purple to yellow. The number of colour changes in the 96-well plates determined the significance of mutations.

## 2.11. Statistical analysis

Data from haematological and biochemical analyses were expressed as mean ± standard error of the mean (mean ± SEM). One-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test was used to determine differences between groups.  $P < 0.05$  was deemed statistically significant. The duplication method was used before actual analysis of the micro and macro-elements. The method employs three-axial geometry, thereby reducing background noise by radiation polarisation. For the Ames test, a statistical table provided in the Muta-ChromoPlate™ two strain kit was used to compare the natural background rate of reverse mutation to the rate of reverse mutation within an assay. Based on these,  $p$  values of 0.001 were classified as highly mutagenic,  $p$  values of 0.01 moderately mutagenic and  $p$  values of 0.05 weakly mutagenic. All the statistical analyses and data presentations were done using Graphpad InStatVersion 3.06 (GraphPad Software, Inc. La Jolla, Ca, USA) and Microsoft Excel 2013 (Microsoft

Corp., Redmond, WA, USA).

## 3. Results

### 3.1. HPLC fingerprint

HPLC fingerprint of *T. schimperi* showed 2 peaks. The most prominent peak had a retention time of 2.5 min. However, the exact compound for this peak is yet to be characterised. HPLC chromatogram of *T. schimperi* is shown in Fig. 1.

### 3.2. Macro- and micro-elemental constituent of TSK

A total of 8 macro-elements and 10 micro-elements were identified and quantified in the aqueous extract of TSK. Macro-elements identified with mean values between 102.33 and 275,315.67 ppm were silicon (Si), aluminium (Al), potassium (K), iron (Fe), titanium (Ti), sulphur (S), zirconium (Zr) and rubidium (Rb). Micro-elements with mean values between 3 and 38 ppm were chromium (Cr), strontium (Sr), nickel (Ni), silver (Ag), yttrium (Y), arsenic (As), zinc (Zn) and niobium (Nb). Magnesium (Mg), phosphorus (P), calcium (Ca), copper (Cu), cadmium (Cd), vanadium (V), thorium (Th) and uranium (U) were not detected in the TSK samples analysed. The levels of elements quantified in the TSK extract were in the order: Si > Al > K > Fe > Ti > S > Zr > Rb > Cr > Sr > Ni > Ag > Y > As > Zn > Nb. The macro and micro-elemental constituent of TSK are presented in Table 1.

### 3.3. Acute toxicity of the aqueous extract of TSK

Oral administration of a single dose of the aqueous extract of TSK (5000 mg/kg) did not cause any death in the SD rats during the study period. The LD<sub>50</sub> of TSK extract was thus established to be higher than 5000 mg/kg. The oral administration of single doses of the aqueous extract of TSK (1000 and 3000 mg/kg) did not show any observable physical signs of toxicity (mobility, salivation, sleep, lethargy, piloerection, and tremor) in the SD rats. Body weight of SD rats each week and weight of isolated organs are presented in Table 2 (a & b). Results showed that aqueous extract of TSK at all doses used for acute toxicity assessment did not cause any significant change in body and organ weight of SD rats.

Results of haematology did not vary significantly among groups. However, MCV and MCHC levels increased significantly in groups treated with 1000 and 3000 mg/kg of the extract of TSK ( $p < 0.05$  and 0.001, respectively) compared to the control group (Table 3).

ALT level was significantly high ( $p < 0.05$ ) in rats administered 1000 and 3000 mg/kg of TSK compared to control. Serum creatinine level was significantly low ( $p < 0.001$ ) in SD rats administered 1000 and 3000 mg/kg of TSK compared to the control group (Table 4). Differences in haematological and biochemical parameters among various groups were not dose-dependent.

Histopathological examination of organs revealed no significant alterations in organs of SD rats in various groups (Fig. 2). Sections of isolated heart from rats administered extracts of TSK showed normal musculature with well-branched myocardial fibres and centrally placed nuclei. Sections of kidneys in rats administered TSK showed normal glomeruli, tubules and interstitium. The liver sections showed hexagonal arrangement of hepatocytes in lobules surrounding a central vein. The sections of spleen had normal lymphoid tissues with appropriate distinction between red and white pulps. The lungs had normal alveolar sac, respiratory bronchioles, and interstitial infiltration of lymphocytes. However, a few mildly congested vessels (with no lymphocyte infiltration and no hepatocellular necrosis) were found in the lungs of all SD rats in various groups.

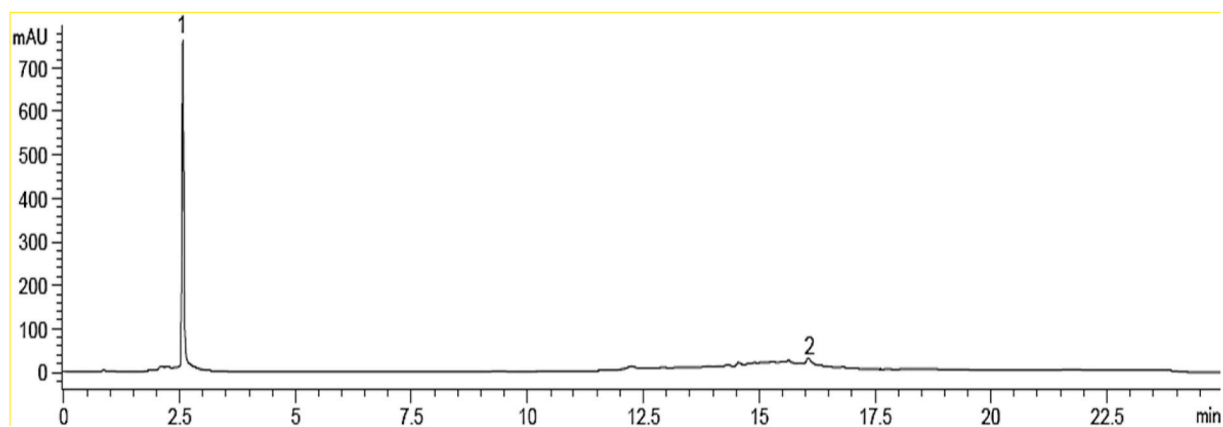


Fig. 1. Chromatogram of HPLC fingerprint of *T. schimperi* that shows two peaks (peak 1 and 2) obtained by C18 (5  $\mu\text{m}$ ) chromatographic column.

Table 1

Content and limit of detection (ppm) of micro and macro-elements in *Termitomyces schimperi* and kaolin (TSK) mixture.

Elements	Mean $\pm$ 3 $\sigma$ (ppm)	Limit of detection (LOD in ppm)
Si	275,315.67 $\pm$ 0.0218	160
Al	123,109.67 $\pm$ 0.0328	95
K	22,089.33 $\pm$ 0.0532	16
Fe	4359.67 $\pm$ 0.01494	12
Ti	3545.67 $\pm$ 0.1013	25
S	273.33 $\pm$ 0.1811	13
Zr	123.33 $\pm$ 0.0702	1
Rb	102.33 $\pm$ 0.0448	1
Cr	38.00 $\pm$ 0.8242	8
Sr	32.33 $\pm$ 0.0536	1
Ni	25.00 $\pm$ 0.2400	4
Ag	17.67 $\pm$ 0.2593	2
Y	17.33 $\pm$ 0.1999	1
As	9.67 $\pm$ 0.1791	1
Zn	9.33 $\pm$ 0.4912	1
Nb	7.00 $\pm$ 0.4286	1
V	ND	4
Th	ND	2
Mg	ND	1100
P	ND	10
Ca	ND	17
Cu	ND	2
Cd	ND	2
U	ND	1

Measurements were done in triplicate, and data presented are expressed in ppm and are mean values  $\pm$  3 $\sigma$ . The limit of detection for light elements (Si, Al, Mg, and Na) was in the range of 25–50 ppm and 1–5 ppm for heavy metals. ND (not detected) means elements probably present but below the limit of detection.

Table 2a

Weekly mean ( $\pm$ SEM) weights (in grams) of rats during the acute toxicity experiment (n = 5 from the beginning of the experiment till the end).

Groups Days	Control (distilled water)	TSK <sub>1000</sub> mg/kg	TSK <sub>3000</sub> mg/kg	TSK <sub>5000</sub> mg/kg	p-value 1
1	138.3 $\pm$ 9.83	151.5 $\pm$ 9.22	158.7 $\pm$ 10.57	144.1 $\pm$ 10.05	0.5103
7	139.2 $\pm$ 9.84	152.4 $\pm$ 9.20	159.6 $\pm$ 10.70	144.8 $\pm$ 10.07	0.5126
14	132.0 $\pm$ 12.10	145.8 $\pm$ 9.58	155.6 $\pm$ 10.32	143.8 $\pm$ 9.03	0.4738
p-value 2	0.8769	0.8651	0.9607	0.9971	–

Values are expressed as mean  $\pm$  SEM. P-value 1 represents comparison I after one-way ANOVA between groups. P-value 2 represents comparison after one-way ANOVA for time-course assessment within groups.

Table 2b

Organ/body weight ratio of rats in the acute toxicity experiment (n = 5 at the end of the experiment).

Organ	Organ/Body weight Ratio ( $1 \times 10^{-3}$ )			
	Control (distilled water)	TSK 1000 mg/Kg	TSK 3000 mg/Kg	TSK 5000 mg/Kg
Lungs	8.897 $\pm$ 2.178	9.503 $\pm$ 1.712	8.275 $\pm$ 0.7894	9.275 $\pm$ 1.226
Heart	4.105 $\pm$ 0.1525	4.244 $\pm$ 0.5507	4.111 $\pm$ 0.5641	4.118 $\pm$ 0.4729
Kidneys	7.682 $\pm$ 1.209	7.796 $\pm$ 0.6016	7.224 $\pm$ 0.5526	7.207 $\pm$ 0.5061
Liver	45.57 $\pm$ 12.57	49.88 $\pm$ 3.400	51.11 $\pm$ 4.344	44.84 $\pm$ 2.684
Spleen	2.257 $\pm$ 0.1253	2.084 $\pm$ 0.2251	2.335 $\pm$ 0.4302	2.379 $\pm$ 0.3934

#### 3.4. Sub-chronic toxicity of the aqueous extract of TSK

Repeated daily administration of the aqueous extract of TSK (200 and 500 mg/kg) over 90 days did not cause any significant change in physical signs (mobility, salivation, sleep, lethargy, piloerection, and tremor) or mortality. The mean (weekly) body weight of rats administered 200 and 500 mg/kg of TSK showed no statistically significant differences ( $p > 0.05$ ) compared to the control group (Table 5a). A significantly higher weight was observed in the lungs and testes of rats administered 500 mg/kg compared to the control group (Table 5b). However, the other harvested organs showed no statistically significant differences in weight. All haematological (Table 6) and biochemical (Table 7) parameters measured were not significantly different ( $p > 0.05$ ) among groups administered TSK compared to control. Histological examination of the organs (Fig. 3) showed no significant structural distortions in the heart, kidney, spleen, lungs, testes, and liver of rats administered aqueous extract of TSK. In the rats administered 500 mg/kg of TSK extract, the liver showed a few dilated central veins which were not found in other two groups. A few congested vessels with lymphocyte infiltration were found in the lungs of all TSK-administered groups. Histology of the sectioned lungs of the control group also exhibited similar features.

#### 3.5. Mutagenic potential of the aqueous extract of TSK

In the presence of 10000  $\mu\text{g/mL}$  of the extract of TSK, it was observed that the TA98 bacteria grew and formed colonies. The mutagenic ability of TSK extract was determined to be weak compared to the positive control ( $p < 0.05$ ). However, in the presence of metabolic activation, TSK extract exhibited high mutagenic activity ( $p < 0.001$ ). Results of the mutagenic potential of the aqueous extract of TSK are presented in

**Table 3**

Haematological parameters after single-dose administration of *T. schimperi* and kaolin (TSK) at 1000; 3000 and 5000 mg/kg during acute toxicity experimentation in male SD rats.

Groups Parameters	Control (distilled water)	TSK <sub>1000</sub> mg/kg	TSK <sub>3000</sub> mg/kg	TSK <sub>5000</sub> mg/kg	p-value
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	5.40 ± 1.00	7.14 ± 2.13	7.58 ± 2.13	7.66 ± 0.75	0.7423
RBC (10 <sup>6</sup> /mm <sup>3</sup> )	7.07 ± 0.32	6.69 ± 0.37	6.19 ± 0.68	7.24 ± 0.16	0.0773
HGB (g/dl)	13.38 ± 0.37	13.26 ± 0.48	12.24 ± 1.16	13.66 ± 0.33	0.0451
HCT (%)	33.96 ± 1.35	36.10 ± 2.63	26.98 ± 2.94	32.38 ± 0.65	0.0511
MCV (µm <sup>3</sup> )	48.00 ± 1.05	54.20 ± 3.26*	43.60 ± 0.68	44.80 ± 0.49	0.0014
MCHC (g/dl)	39.54 ± 0.61	37.28 ± 2.20	45.80 ± 1.14**	42.22 ± 0.37	0.0099
PLT (10 <sup>3</sup> /mm <sup>3</sup> )	805.40 ± 119.10	769.40 ± 50.27	645.20 ± 102.00	819.20 ± 104.80	0.4703
LYM (%)	65.52 ± 7.55	59.60 ± 3.36	57.84 ± 2.35	58.22 ± 5.53	0.1545
LYM (10 <sup>3</sup> /mm <sup>3</sup> )	3.58 ± 0.92	4.04 ± 1.17	4.36 ± 1.25	4.38 ± 0.56	0.9369
RDW (%)	14.90 ± 0.21	15.08 ± 0.59	14.66 ± 0.50	14.68 ± 0.12	0.0433
PDW (%)	5.74 ± 1.28	10.70 ± 2.70	2.50 ± 1.02	3.48 ± 1.02	0.4764
MPV (µm <sup>3</sup> )	6.92 ± 0.64	7.70 ± 0.51	7.70 ± 0.29	6.92 ± 0.25	0.2605
GRA (10 <sup>3</sup> /mm <sup>3</sup> )	1.26 ± 0.37	2.40 ± 0.83	2.18 ± 0.69	2.26 ± 0.41	0.3711
GRA (%)	22.62 ± 6.28	28.82 ± 3.93	27.10 ± 3.62	27.36 ± 4.40	0.7052

Values are expressed as mean ± SEM (n = 5). P value represents significance level for one-way ANOVA (followed by Newman-Keuls Multiple Comparison Test) with \* indicating significant difference from the control (distilled water) \* = P < 0.05 and \*\* = P < 0.01 and \*\*\* = P < 0.001.

**Table 8.**

#### 4. Discussion

Mushrooms are widely used in the management of a number of diseases (Hsieh and Ju, 2018). A survey conducted in Abidjan (Cote d'Ivoire) revealed that *T. schimperi* was used in combination with kaolin to manage cancers in patients. Due to the potential of some mushrooms to be poisonous (Govorushko et al., 2019), we sought to assess the safety of TSK through *in vitro* and animal model toxicity experiments. The present study reports acute and sub-chronic toxicities, mutagenic potential, and trace metal constituent of TSK for the first time.

We also report for the first time HPLC chromatogram of *T. schimperi*. The chromatogram showed one prominent peak at a retention time of 2.5 min, which may be an indication of high levels of a particular compound yet to be identified. It can be postulated that this compound may be responsible for the pharmacological (anticancer) activity of TSK. Nonetheless, compounds present at low concentrations in any natural product may also exert more potent biological activity than those present at higher concentrations (Cieřla, 2012). The HPLC fingerprint presented may be relevant for future quality control assessments of *T. schimperi* (Su et al., 2007; World Health Organization Programme on Traditional, 2000).

With the lethal dose and fixed-dose studies (acute toxicity) in SD rats, high doses of TSK were administered, and physical signs and/or mortality were monitored for 14 days. Results showed that oral administration of the aqueous extract of TSK did not cause any death among the SD rats. It could be inferred from the results that the LD<sub>50</sub> of TSK extract was above 5000 mg/kg. This showed that TSK may be non-toxic. Additionally, this acute toxicity test revealed that the oral

**Table 4**

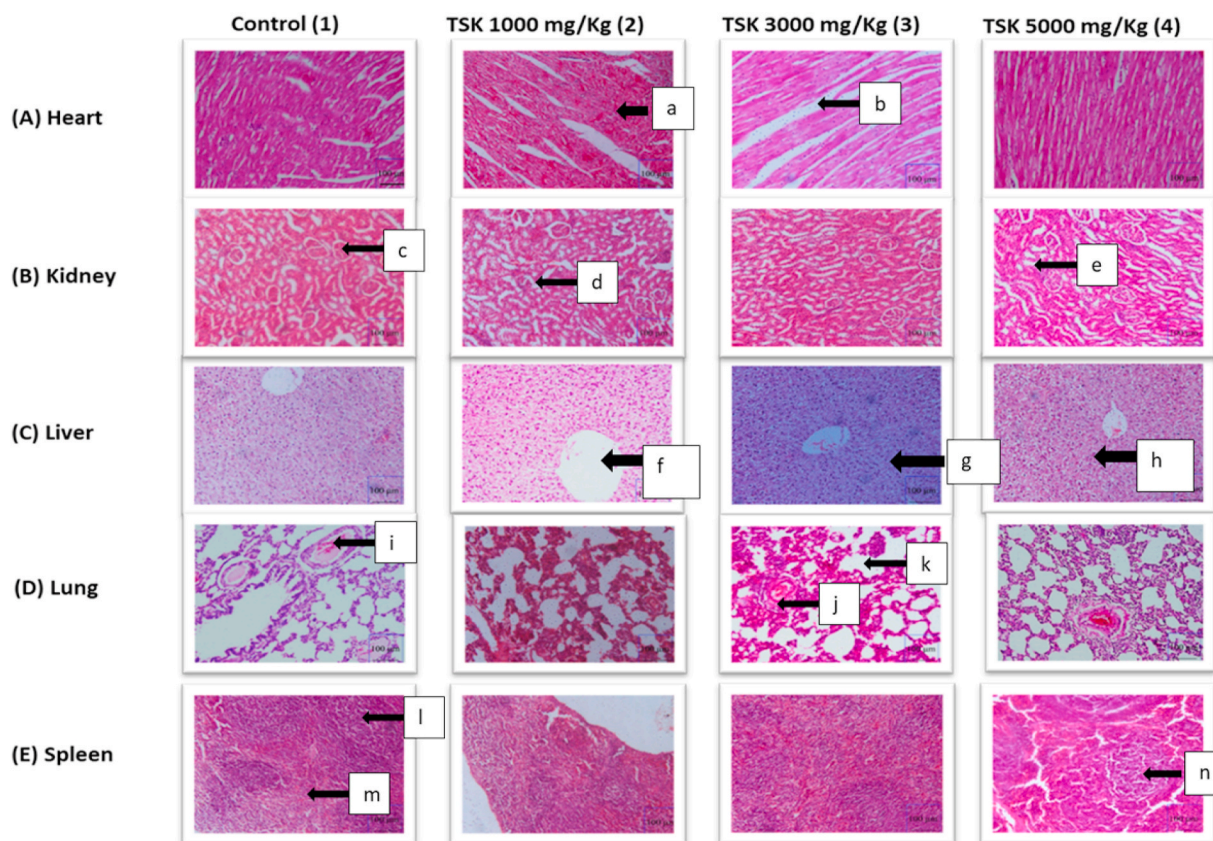
Blood biochemistry after administration of single doses of *T. schimperi* and kaolin (TSK) at 1000; 3000 and 5000 mg/kg in SD male rats during acute toxicity experimentation.

Group Parameters	Control (distilled water)	TSK <sub>1000</sub> mg/kg	TSK <sub>3000</sub> mg/kg	TSK <sub>5000</sub> mg/kg	p-value
Urea (mmol/L)	7.75 ± 0.82	6.40 ± 0.57	7.65 ± 2.21	5.59 ± 1.01	0.6395
Serum creatinine (mmol/L)	43.10 ± 1.32	37.48 ± 0.58*	37.16 ± 0.83*	39.54 ± 1.62	0.0122
ALT (U/L)	87.60 ± 3.57	143.70 ± 14.84**	175.60 ± 11.67***	91.68 ± 2.63	<0.0001
AST (U/L)	233.10 ± 24.85	294.40 ± 46.38	329.80 ± 71.76	230.70 ± 6.59	0.3826
ALP (U/L)	832.40 ± 171.00	885.80 ± 80.30	990.10 ± 132.20	715.20 ± 68.03	0.3946
Total-Bilirubin (µmol/L)	1.02 ± 0.45	0.38 ± 0.31	0.99 ± 0.34	0.93 ± 0.17	0.4543
Direct-Bilirubin (µmol/L)	8.91 ± 2.96	12.35 ± 5.10	15.64 ± 6.16	6.14 ± 0.34	0.4614
Indirect-Bilirubin (µmol/L)	-7.87 ± 2.62	-11.96 ± 4.92	-14.66 ± 6.21	-5.22 ± 0.39	0.4333
Total protein (g/L)	61.25 ± 2.95	59.20 ± 2.69	60.80 ± 2.92	61.60 ± 1.29	0.9066
Albumin (g/ml)	11.75 ± 6.78	100.00 ± 41.74	29.20 ± 27.72	3.80 ± 3.07	0.0781
Total-Cholesterol (mmol/L)	2.08 ± 0.23	2.12 ± 0.14	2.15 ± 0.18	2.06 ± 0.14	0.9831
Triglycerides (mmol/L)	1.36 ± 0.08	1.31 ± 0.18	1.56 ± 0.28	1.51 ± 0.28	0.7573
HDL-Cholesterol (mmol/L)	1.34 ± 0.15	1.18 ± 0.10	1.20 ± 0.13	1.08 ± 0.08	0.5506
LDL-Cholesterol (mmol/L)	0.13 ± 0.08	0.33 ± 0.19	0.25 ± 0.11	0.29 ± 0.08	0.7371
Glucose (mmol/L)	8.58 ± 0.80	9.01 ± 0.97	9.58 ± 0.50	7.89 ± 0.68	0.4503
Globulin (g/L)	49.50 ± 6.36	40.80 ± 40.37	31.60 ± 29.52	57.80 ± 3.40	0.0686

Values are expressed as mean ± SEM (n = 5). P-value represents significance level after one-way ANOVA (followed by Newman-Keuls Multiple Comparison Test) with \* indicating significant difference compared to control (distilled water) \* = P < 0.05 and \*\* = P < 0.01 and \*\*\* = P < 0.001.

administration of the aqueous extract of TSK at all doses did not cause any significant change in body and organ weights of the rats. Results from sub-chronic toxicity assessment also did not show significant change in the survival, mean body and organ weights of the SD rats. However, the weight of the lungs and testes of SD rats administered TSK extract showed some level of change after the 90-day period. This change in organ weight, however, was not found to be dose-dependent.

Data from the present study showed no statistically significant differences in blood parameters of SD rats administered extracts of TSK. However, increases in MCV and MCHC were observed compared to control. It is noteworthy, that these differences observed in SD rats administered extracts of TSK were not dose-dependent. Generally, MCV and MCHC results tend to mirror each other and are relevant indicators in anaemia diagnosis (Weingand et al., 1996). A low MCHC indicates iron-deficit anaemia, while high MCHC indicates anaemia due to vitamin B deficiency. These results could suggest macrocytic anaemia; but this hypothesis is not supported by other measured parameters, RBC and haemoglobin which did not change significantly compared to control. Hence, the increase in MCV and MCHC could be of no toxicological relevance. Additionally, the values obtained were within normal laboratory reference ranges (MCV: 48.9–57.9; MCHC: 32.9–52.5) (Clifford



**Fig. 2.** Photomicrographs (H&E Staining 40x) of the heart (A), kidney (B), liver (C), lung (D) and spleen (E) isolated after 14-day administration of single doses of distilled water (1), TSK extract at 1000 mg/kg (2), 3000 mg/kg (3) and 5000 mg/kg (4). (a) shows myocardium with (b) intercalated discs. (c) shows glomerulus with (d) proximal tubule and (e) distal tubule. (f) shows a slightly dilated vein and (g) which shows normal hepatocytes with (h) normal central veins. (i) shows a few congested vessels and patches of leucocytes infiltration with (j) showing the bronchiole and (k) showing the alveoli. (l) shows white pulp and (m) shows red pulp and (n) shows central arteries.

and Giknis, 2008).

Liver enzymes (AST, ALT, and ALP) and kidney biomarkers (urea, total protein and creatinine) were assayed and used as indices of toxicity in these organs (Mukinda and Syce, 2007). Our findings from acute toxicity study showed that the level of ALT was significantly high, while serum creatinine level was significantly low in SD rats administered extracts of TSK. These changes in ALT and creatinine levels in rats administered extracts of TSK were not dose-dependent. Low levels of serum creatinine could be associated with a decrease in muscle mass. Furthermore, high levels of ALT could be an indication of cirrhosis, liver tumour, liver tissue death, or lack of blood-flow to liver (Hall et al., 2012). Usually, ALT is compared with albumin, AST, alkaline phosphatase, bilirubin, and total protein. AST tends to increase with ALT if there is significant liver damage (Bridges-Webb, 2008). Other hepatic parameters (albumin, total protein, globulin, total bilirubin, direct bilirubin, indirect bilirubin, AST, and ALP) did not vary significantly. Therefore, changes in ALT observed in this study may not be clinically relevant. In the sub-chronic toxicity study, aqueous extract of TSK did not cause any significant change in biochemical parameters. This suggests that the extract of TSK did not have any toxic effect on the kidney and liver.

Histopathological examination of some selected organs showed no major pathophysiological changes. However, the lungs of SD rats administered extracts of TSK showed few congested vessels and leucocyte infiltration patches. These changes observed in the lungs were also found in rats that served as control. Congested vessels and few patches of leucocytes infiltration in the lungs could be due to repeated airway exposure to wood dust released from softwood shavings used as bedding (Lee, 2016; Määttä et al., 2006). Histological assessment of liver sections

from SD rats after administration of TSK extract for 90 days showed that the liver had mildly dilated central veins. However, this structural change was not associated with hepatocellular necrosis or leucocyte infiltration. Sinusoidal dilation, pericellular/sinusoidal fibrosis and fibrosis surrounding the central vein are often deemed pathological. The aforementioned are more common in congestive hepatopathy (Gonzalez et al., 2016). Sinusoidal dilation without necrosis has been associated with heart failure and systemic venous pressure. Since this finding had no direct link with a pathological finding from the heart, this alteration cannot be attributed to the TSK extract. Also, the histopathological assessment did not show any pathophysiological changes in the testes of the SD rats. Therefore, the doses of TSK extracts tested were within the no-observed adverse effects level (NOAEL).

Earlier studies estimating the toxicity potential of *T. robustus* (Ugbogu et al., 2019) and *T. albuminosus* (An and Park, 2017) reported that these *Termitomyces* species were not toxic and were safe for consumption. On the contrary, *T. clypeatus* exhibited toxic and teratogenic effects on zebrafish embryos (De Castro et al., 2016) and *T. striatus* was also found to cause death in rats on the fourth day following administration (Adewusi et al., 1993). Also, some studies have shown that kaolin alleviates toxicity of compounds such as graphene oxide, lead, arsenic (Rozhina et al., 2019; Wapnir et al., 1980; Zhou et al., 2010) and may be consistent with our findings of the combination *T. shimperi* and kaolin.

In addition, we tested the mutagenic potential of the aqueous extract of TSK on *S. typhimurium* TA98. These bacteria are mutant strains developed by Ames (Ames et al., 1975). They cannot synthesise histidine and form colonies in a histidine-free medium unless a spontaneous mutation (a rare event) or a mutation caused by the tested agent reverts its genotype to the wild-type. Our results showed that in the presence of

**Table 5a**

Mean ± SEM weights of rats (n = 6) during 90-day sub-chronic toxicity assessment of TSK.

Groups Weeks	Control (distilled water)	TSK <sub>200</sub> mg/kg	TSK <sub>500</sub> mg/kg	p-value 1
1	143.00 ± 8.04	154.40 ± 10.07	133.90 ± 5.28	0.2283
2	148.90 ± 10.83	177.10 ± 12.21	136.60 ± 10.46	0.0592
3	142.90 ± 10.61	178.10 ± 12.21*	136.00 ± 6.61	0.0216
4	151.80 ± 13.37	189.90 ± 1.34	155.60 ± 8.79	0.0778
5	164.10 ± 12.82	200.00 ± 14.14	162.10 ± 9.62	0.0813
6	172.50 ± 10.60	188.70 ± 13.74	169.60 ± 8.65	0.4531
7	196.20 ± 9.99	196.60 ± 13.09	190.00 ± 8.72 <sup>b</sup>	0.8823
8	188.60 ± 10.71	185.00 ± 14.78	188.80 ± 4.39 <sup>b</sup>	0.9616
9	191.10 ± 16.58	205.10 ± 14.76	161.20 ± 8.14	0.1015
10	211.60 ± 16.54 <sup>a</sup>	210.70 ± 15.36	175.40 ± 13.46	0.1869
11	221.60 ± 17.59 <sup>b</sup>	223.90 ± 16.78 <sup>a</sup>	192.10 ± 13.97 <sup>b</sup>	0.3228
12	222.60 ± 15.26 <sup>b</sup>	221.00 ± 16.50	207.20 ± 16.24 <sup>c</sup>	0.7507
13	223.40 ± 14.24 <sup>b</sup>	221.10 ± 15.36	201.60 ± 12.63 <sup>b</sup>	0.5156
<b>P value 2</b>	<0.0001	0.0261	<0.0001	-

Values are expressed as mean ± SEM. P-value 1 represents significance level for one-way ANOVA (followed by Newman-Keuls Multiple Comparison Test) between-group comparisons. P-value 2 represent significance level for one-way ANOVA (followed by Newman-Keuls Multiple Comparison Test) for time within groups. Alphabets indicate significant difference from week 1 to week 13. a = P < 0.05 and b = P < 0.01 and c = P < 0.001.

**Table 5b**

Organ/body weight ratio of rats in the sub-chronic toxicity experiment (n = 6 at the end of the experiment).

Organ	Organ/Body weight Ratio (1 × 10 <sup>-3</sup> )		
	Control (distilled water)	TSK <sub>200</sub> mg/kg	TSK <sub>500</sub> mg/kg
Lungs	6.417 ± 0.9615	8.874 ± 1.840	9.501 ± 2.701*
Heart	3.492 ± 0.4178	3.449 ± 0.8895	4.291 ± 0.7925
Kidneys	3.621 ± 0.4372	2.925 ± 0.4054	3.797 ± 0.8022
Liver	28.67 ± 4.453	28.27 ± 1.074	30.53 ± 3.054
Spleen	1.892 ± 0.3320	2.198 ± 0.6819	1.754 ± 0.2225
Testes	8.278 ± 1.620	8.218 ± 1.962	10.90 ± 0.6513*

the aqueous extract of TSK, TA98 bacteria were able to grow and form colonies. Therefore, it could be inferred that TSK extract had measurable mutagenic ability. This was, however, classified as weak when compared to the positive control. A majority of mutagenic xenobiotics require bioactivation (toxification) by reactive electrophiles to induce deoxyribonucleic acid (DNA) damage. Our finding showed that in the presence of liver extract S9 (which contains the necessary cofactors and numerous exogenous metabolic systems), the aqueous extract of TSK exhibited high mutagenic activity. A mutation restoring the ability of TA98 strain to synthesise histidine is categorised as frameshift mutation. Our results corroborate other studies that have reported potential genotoxic effect of kaolin (Maisanaba et al., 2015; Totsuka et al., 2009). Also, the mutagenic potential of TSK could be consistent with its traditional use as an anticancer agent. Some anticancer drugs, mutagenic in nature, are currently used in chemotherapy (Ferguson and Pearson, 1996; Parrella et al., 2015). However, further studies are required to confirm preliminary findings on the genotoxic potential of the aqueous extract of TSK.

**Table 6**

Haematological analysis of daily single administration of *T. shimperi* and kaolin (TSK) at 200 and 500 mg/kg after a 90-day administration in SD male rats.

Groups Parameters	Control (distilled water)	TSK <sub>200</sub> mg/kg	TSK <sub>500</sub> mg/kg	p-value
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	10.65 ± 1.01	16.52 ± 3.31	16.02 ± 5.19	0.4009
RBC (10 <sup>6</sup> /mm <sup>3</sup> )	7.31 ± 0.46	7.52 ± 0.21	7.88 ± 0.19	0.4947
HGB (g/dl)	14.73 ± 0.22	14.12 ± 0.63	15.06 ± 0.32	0.3121
HCT (%)	46.70 ± 0.88	43.30 ± 1.57	47.50 ± 1.23	0.0749
MCV (µm <sup>3</sup> )	65.50 ± 4.95	57.60 ± 0.81	60.40 ± 1.03	0.2587
MCHC (g/dl)	31.47 ± 0.52	32.54 ± 0.43	31.72 ± 0.34	0.2531
PLT (10 <sup>3</sup> /mm <sup>3</sup> )	523.90 ± 158.50	700.40 ± 75.21	744.80 ± 61.82	0.3764
LYM (10 <sup>3</sup> /mm <sup>3</sup> )	11.36 ± 1.83	10.00 ± 1.95	9.96 ± 1.72	0.8307
RDW (%)	16.58 ± 0.83	15.16 ± 0.18	16.28 ± 0.36	0.2345
PDW (%)	10.50 ± 0.74	8.68 ± 1.57	8.92 ± 0.75	0.4221
MPV (µm <sup>3</sup> )	6.99 ± 1.28	8.70 ± 0.32	8.62 ± 0.21	0.3073
GRA (10 <sup>3</sup> /mm <sup>3</sup> )	3.46 ± 1.17	4.42 ± 1.38	3.56 ± 1.55	0.8648

Values are expressed as mean ± SEM (n = 6). P-value represents significance level for one-way ANOVA (followed by Newman-Keuls Multiple Comparison Test).

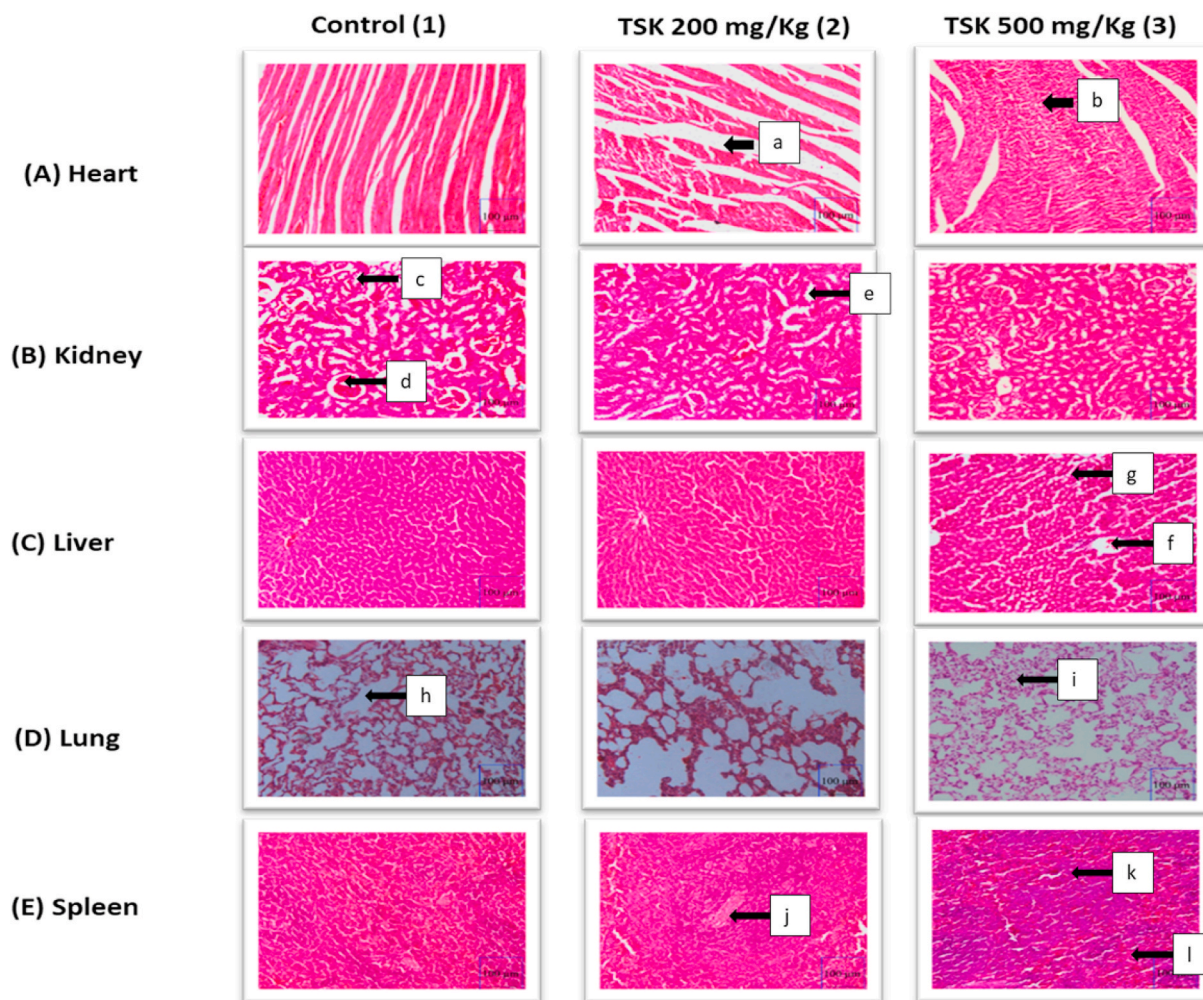
**Table 7**

Blood biochemistry after a daily single administration of *T. shimperi* and kaolin (TSK) at 200 and 500 mg/kg over 90 days in SD male rats.

Groups Parameters	Control (distilled water)	TSK 200 mg/kg	TSK 500 mg/kg	p-value
Urea (mmol/L)	7.79 ± 0.62	6.66 ± 0.51	6.65 ± 0.65	0.3340
serum creatinine (mmol/L)	37.26 ± 0.65	37.06 ± 0.88	38.08 ± 1.79	0.8230
ALT (U/L)	126.20 ± 11.02	134.20 ± 19.80	135.80 ± 17.64	0.9096
AST (U/L)	248.70 ± 13.03	256.40 ± 20.43	241.50 ± 33.10	0.9070
ALP (U/L)	713.60 ± 68.67	774.20 ± 56.52	758.10 ± 54.47	0.7666
Total-Bilirubin. (µmol/L)	0.74 ± 0.21	1.40 ± 0.23	1.12 ± 0.33	0.2591
Direct-Bilirubin (µmol/L)	13.07 ± 3.93	13.11 ± 4.10	13.40 ± 4.28	0.9981
Indirect-Bilirubin (µmol/L)	-12.70 ± 6.25	-10.78 ± 3.41	-10.94 ± 4.10	0.9510
Total protein (g/L)	57.00 ± 2.63	61.60 ± 2.27	63.20 ± 2.74	0.2449
Albumin (g/ml)	66.60 ± 35.52	42.00 ± 36.92	64.20 ± 39.84	0.8784
Total-Cholesterol (mmol/L)	2.06 ± 0.17	1.91 ± 0.16	2.03 ± 0.19	0.8036
Triglycerides (mmol/L)	1.57 ± 0.30	1.56 ± 0.21	1.81 ± 0.25	0.7396
HDL-Cholesterol (mmol/L)	1.230 ± 0.150	1.158 ± 0.092	1.108 ± 0.094	0.7605
LDL-Cholesterol (mmol/L)	0.402 ± 0.159	0.298 ± 0.095	0.346 ± 0.0287	0.7972
Glucose (mmol/L)	7.742 ± 0.700	9.036 ± 0.812	8.610 ± 0.978	0.5547
Globulin (g/L)	63.60 ± 7.284	50.00 ± 5.099	59.00 ± 1.871	0.2172

Values are expressed as mean ± SEM (n = 6). P-value represents significance level for one-way ANOVA (followed by Newman-Keuls Multiple Comparison Test).

Mushrooms are known for their ability to readily absorb heavy metals from the environment compared to green plants (Melgar et al., 2016; Türkmen and Budur, 2018). The current study estimated trace metal constituents as part of toxicological assessment of the extract of TSK. Results from the current study revealed the presence of 8 macro-elements and 8 micro-elements. Levels of trace metals found in



**Fig. 3.** Photomicrographs (H&E Staining 40x) of the heart (A), kidney (B), liver (C), lung (D) and spleen (E) isolated after a 90-day continuous oral administration of distilled water (1), TSK extract at 200 mg/kg (2) and 500 mg/kg (3). (a) shows intercalated discs with (b) myocardium. (c) shows glomerulus with (d) proximal tubule and (e) distal tubule. (f) shows a mildly dilated vein and (g) which shows normal hepatocytes. (h) shows alveoli with (i) showing the bronchiole. (j) shows the central arteries and (k) shows the white pulp and (l) shows the red pulp. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 8**

Ames assay of TSK samples (10,000 µg/mL) after 5-day incubation with TA 98 strain.

Samples		p-value	Results	Interpretation
Positive control	Nitroflourene	0.001	Highly mutagenic	Frameshift mutation before and after metabolism
	Nitroflourene + S9	0.001	Highly mutagenic	
<i>T.schimperi</i> /Kaolin (TSK)	TSK	0.05	Weakly mutagenic	Frameshift mutation before and after metabolism
	TSK + S9	0.001	Highly mutagenic	

the TSK extract, from highest to the lowest were; Si, Al, K, Fe, Ti, S, Zr, Rb, Cr, Sr, Ni, Ag, Y, As, Zn, and Nb. Heavy metals known to be hazardous include As, Cd and U (Tchounwou et al., 2012; Türkmen and Budur, 2018; Wang et al., 2017). Others like Fe, Cu, Cr and Zn are considered less harmful (Govorushko et al., 2019). Results from this study showed absence of Cd and U in the extract of TSK. Our findings in the acute and sub-chronic toxicity studies may be consistent with the absence of Cd and U in TSK extract. The mutagenic potential of TSK may be associated with Al, Ti, Zr and Rb, and, to a lesser extent, the presence

of Ag. Results from current study showed that the TSK extract did not contain Mg, P, Ca, Cu, V, and Th. These findings contradict literature which suggests that mushrooms are known to contain Ca, Mg, and P (in addition to K) (Mallikarjuna et al., 2013). The difference in elemental constituents between the current study and others could be attributed to specie differences and geographical location of mushrooms.

**5. Conclusions**

Data from the current study showed that oral administration of single (high) doses of aqueous extract of TSK did not cause any significant change in the body weight, haematological, biochemical, and histological parameters of SD rats after 14 days. A similar trend was observed after administration of repeated doses of extract of TSK over 90 days. Furthermore, measurable mutagenic potential of the aqueous extract of TSK was observed on *S. typhimurium* TA98 strain, and this could be attributed to the presence of toxic and potentially toxic trace elements in TSK. Further studies (chronic toxicity and additional mutagenic assays) are, however, required.

**Authors' contributions**

BBN conceptualised the research. BBN, SKA, MAS, SFM, EO-A, EOB,

RA-O and IA-G designed the methodology. IBI and EO-A performed experimental work under the supervision of BBN, SKA, MAS, SFM, RA-O and EOB. BBN, SKA, IBI, EO-A and EOB put data together. BBN, SKA, IBI, MAS, SFM, EO-A, EOB, JAS, RA-O and IA-G performed data analysis. BBN, SKA, IBI, MAS, SFM, EO-A, EOB, JAS, RA-O and IA-G were involved in writing and revising the manuscript. All authors read and approved final version of the manuscript.

## Funding

This work did not receive any funding.

## Declaration of competing interest

The authors declare no conflicts of interest.

## Acknowledgments

The authors are grateful to Non-Governmental Organisation for Promotion of Scientific Research in Traditional African Medicine (NGO "PRORESMAT"). We are indebted to the late Prof. Laurent Ake-Assi (Felix Houphouët-Boigny University, Abidjan, Cote d'Ivoire) for introducing Dr Benoit Banga N'guessan to Mrs Adjoa Agnes Koffi. We thank Mrs Koffi for accepting to collaborate with us and for helping in identification and collection of the mushroom and kaolin. We also appreciate Dr Kroa Ehoule, Director of the National Programme for Promotion of Traditional Medicine (Ministry of Health, Cote d'Ivoire); Prof. Innocent Adoubi, Head of Oncology Department (Treichville Teaching Hospital, Abidjan, Cote d'Ivoire) and Director of the National Programme for Fight against Cancer (Ministry of Health, Cote d'Ivoire) for their contributions. We are also thankful to all laboratory technicians at the School of Pharmacy and Noguchi Memorial Institute for Medical Research, University of Ghana.

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