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# Evaluation of acute and sub-acute toxicity profile of 5-methylcoumarin-4β-glucoside in mice

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ABSTRACT

*Vernonia glaberrima* leaves are traditionally used to alleviate bodily pain, skin cancer, and other skin-related disorders. The purpose of the study was to investigate the acute and sub-acute toxicity of 5-methylcoumarin-4β-glucoside, a promising chemotherapeutic agent against colon cancer isolated from the leaves of *Vernonia glaberrima*. 5-methylcoumarin-4β-glucoside was isolated from the methanol leaf extract of *Vernonia glaberrima* following a previously described method. The acute toxicity study involved a two-phase 24 h observation for signs of mortality and toxicity following single oral dose administration of the isolated compound. For the sub-acute study, four groups of mice, averagely aged eight weeks, were administered graded doses of the compound (250, 500 and 1000 mg/kg) or vehicle for 28 days. On the 29th day, the mice were fasted, anesthetized, euthanized, then their blood and tissues were harvested for hematological, biochemical and histopathological evaluations. There were no signs of mortality or moribund status with an increasing dose of up to 5000 mg/kg over a 24 h period in the acute study. Also, there was no evidence of toxicity on the biochemical or hematopoietic systems in the sub-acute study ($p < 0.05$). At the dose of 1000 mg/kg, the mice showed some distorted histology with no corresponding alterations in serum biochemicals. Overall, the results showed that 5-methylcoumarin-4β-glucoside at dosages up to 500 mg/kg is tolerable in mice.

## 1. Introduction

Ascertaining the toxicity profile of a potential drug candidate is indispensable. Acute toxicity is investigated to gain insight into a compound's short-term adverse effects when administered within 24 h. It provides insights on potential target organ(s) of toxicity, safe acute doses for further studies, the potential for toxicity in humans and drug-induced clinical observations [3]. Sub-acute toxicity demonstrates a compound's intrinsic adverse effect following its daily administration for 1–3 months. Sub-acute toxicity investigates the regression or progression of lesions due to daily administration of a drug candidate. It also encompasses the scope of acute toxicity study [5].

Ensuring that a drug candidate is safe cannot be overemphasized during the drug discovery process [6]. Safety becomes more critical when a drug candidate is a chemotherapeutic agent, as they are usually cytotoxic to normal cells [11]. Ensuring the developed drug is a selectively cytotoxic chemotherapeutic agent is therefore crucial. 5-methylcoumarin-4β-glucoside isolated from the leaves of *Vernonia glaberrima* (Welw. Ex O.Hoffm.) is a potential chemotherapeutic agent against colorectal cancer. The compound has demonstrated *in vitro* cytotoxic activity against colon cancer (HT-29) cell lines [1]. Ongoing *in vivo* studies in our lab using albino mice have corroborated the compound's *in vitro* cytotoxic activity (unpublished data). Therefore, there is a need to establish the short and long term toxicity profile of the compound. In

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view of the aforementioned, this study investigated the acute and sub-acute toxicity study of 5-methylcoumarin-4β-glucoside in mice.

## 2. Methods

### 2.1. Chemicals and reagents

Analytical grade solvents, silica gel 60 (70–230 mesh) and Thin layer chromatography (TLC) plates were purchased from Merck (Merck KGaA, Darmstadt, Germany). All reagents and chemicals used were of analytical grade.

### 2.2. Plant collection and processing

We collected fresh *V. glaberrima* leaves in Nasarawa LGA, Nasarawa State, Nigeria. The leaves were validated at the herbarium of the Department of Pharmacognosy, Usmanu Danfodiyo University, Sokoto, Nigeria. For future reference, a voucher specimen was deposited. Prior to usage, the leaves were air-dried, crushed, and stored in an airtight plastic bag at 4 °C.

### 2.3. Animal handling

Forty albino mice (weighing 20 – 28 g) were purchased from the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja and transferred to the Animal house facility at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto where the animals were maintained throughout the period of the study. The animals were housed in groups of three in transparent polycarbonate cages and kept at 25–30 °C in a well-ventilated room with a 12/12 h light/dark cycle. The University's ethical committee requirements for the use of animals, as well as the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978), were followed. Before the experiment began, the animals were allowed to acclimate for two weeks on *ad libitum* mouse chow and free access to water. The University Research and Ethics Committee approved the research protocol and assigned it a reference number UDUS/UREC/2019/019.

### 2.4. Preparation of plant extract

*V. glaberrima* dry leaves (2.5 kg) were pulverized and soaked in methanol (10 L) for 72 h. Using a rotary evaporator, the extract was collected, filtered, and dried at 40 °C. The extraction process was repeated three times, and the various extracts were combined to yield 220 g of dark green crude methanolic extract.

### 2.5. Isolation of 5-methylcoumarin-4β-glucoside

The isolation was carried out following the method earlier described by Alhassan et al. [1]. Crude methanolic extract (200 g) was suspended in distilled water and sequentially extracted with hexane, chloroform, and n-butanol. The remaining aqueous portion was filtered, and the water-insoluble residue was collected, dried and weighed to yield 30.5 g of a semi-purified compound. Open column chromatography was used to further purify the semi-purified compound. The column was eluted in gradient mode using chloroform: methanol (100:0 -70:30) to yield 50 fractions. The isolation was monitored using thin-layer chromatography. Fractions 12–25 were combined and subjected to crystallization in 95% ethanol to yield 9.7 g of a purified compound. The compound was characterized using melting point, thin layer chromatography and $^{1}$H NMR and $^{13}$C NMR spectroscopy. The melting point was determined using Staurt Scientific Melting Point Apparatus SMP1 (UK). $^{1}$H NMR and $^{13}$C NMR spectra were recorded on Bruker Avance 500 and 600 MHz NMR spectrometers, respectively. TLC plates precoated with silica gel (60 $F_{254}$) were used for thin-layer chromatography.

**Table 1**
Experimental Design of Acute Toxicity Study.

| PHASE ONE (n = 3) | | PHASE TWO (n = 1) | |
|---|---|---|---|
| Route of administration | Dose (mg/kg) | Route of administration | Dose (mg/kg) |
| Oral | 10 | Oral | 1600 |
| Oral | 100 | Oral | 2900 |
| Oral | 1000 | Oral | 5000 |

### 2.6. Acute toxicity study ($LD_{50}$)

The method of Lorke [7] was used to determine the $LD_{50}$ (Table 1). The $LD_{50}$ value was calculated by taking the geometric mean of the lowest dose that caused death to the animal and the highest dose for which the animal survived after 24 h of administering the compound.

### 2.7. Sub-acute toxicity test

Twenty-four mice were used in the sub-acute toxicity research. All the mice were orally administered 5-methylcoumarin-4β-glucoside. The mice were divided into four groups, each containing six mice per group. The first group (vehicle control) received the vehicle at a dose of 2 ml/kg body weight, while the second, third and fourth groups received 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight of 5-methylcoumarin-4β-glucoside respectively. Physiological functions that were monitored included the respiratory rate, occurrence of secretions and excretions, and autonomic activity (such as lacrimation, piloerection, pupil size, and unusual respiratory pattern), changes in skin fur, presence or absence of hair loss (alopecia), eyes and mucous membranes. Behavioral parameters that were observed included changes in gait, posture, and sensitivity to handling, as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling), and bizarre behavior (e.g., self-mutilation, walking backwards) were noted.The mice were weighed weekly to monitor weight changes. They were also monitored for variations in food/water consumption. On day 29 of administration, the mice were fasted for 12 h, euthanized by exposing them to carbon dioxide in a chamber; blood samples were collected by cardiac puncture in plain blood sample tubes. The serum was separated and quantified for glucose, potassium ion, urea, creatinine, uric acid, calcium ion, phosphate, total cholesterol, triglyceride, aspartate aminotransferase, total bilirubin, alkaline phosphatase and alanine transaminase using a Semi Auto Analyser Microlab 300 (Vital Scientific, US) machine and Ion Selective Electrode (ISE) Caretium X1–921 (China) machine. Vital organs of interest, including liver, kidney and colon, were collected after sacrifice. The organs were rinsed with normal saline and examined macroscopically before being placed in RCl2® for further analysis.

### 2.8. Histopathological study

The organs were fixed in a 10% v/v neutrally buffered formaldehyde solution for ten days before being treated with paraffin wax to dehydrate, polish, and infiltrate the tissues. They were then inserted, allowing the specimen to be oriented in a 'block' that can be sectioned and stored and handled easily. It was sectioned with a microtome into very thin slices that were then placed on a microscope slide and stained with hematoxylin and eosin before being examined for histopathological characteristics [12]. A Nikon E-600 microscope was used to take bright-field photomicrographs of the colon of mice using a Retiga 2000R Fast CCD camera (Q-Imaging) (Nikon, Tokyo, Japan).

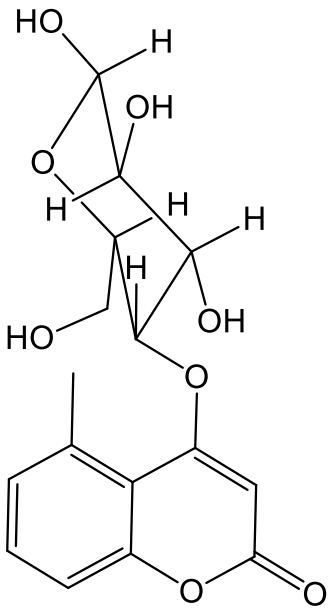


**Fig. 1.** Structure of 5-methylcoumarin-4-β-glucoside.

### 2.9. Statistical analysis

The mean and standard error of the mean (SEM) are used to express the results. SPSS version 19 was used to conduct statistical analysis of the data. As applicable, one-way analysis of variance (ANOVA) or general regression analysis were utilized. For *post hoc* examination of differences found using one-way ANOVA, Tukey's test was utilized. The $p < 0.05$ significance level was chosen. Tables and graphs are used to represent data.

## 3. Results

### 3.1. Isolation and characterization of 5-methylcoumarin-4-β-glucoside

5-methylcoumarin-4-β-glucoside: White crystals, TLC retention factor ($R_f$) value: 4.5 (solvent: hexane/ethyl acetate/ methanol 7:2:1) $^1$H NMR [600 MHz, DMSO-d6, δ (ppm)]: δ 6.0 (1 H, s, H-3); 7.16 (1 H, d, $J$ = 7.6 Hz, H-6); 7.51 (1 H, t, $J$ = 7.8 Hz); 7.24 (1 H, d, $J$ = 8.4 Hz, H-8); 2.71 (3 H, s, H-9); 5.20 ( 1 H, d, $J$ = 7.6 Hz, H-1′); 3.40 (1 H, m, H-2′); 3.35 (1 H, m, H-3′); 3.20 (1 H, td, $J$ = 9.1 Hz, 7.7 Hz, H-4′); 3.50 (1 H, m, H-5′); 3.76 (1 H. m, H-6a′); 3.50 (1 H, m, H-6b′); 5.48 (1 H, d, $J$ = 5.6 Hz, OH-2′); 5.19 (1 H, d, $J$ = 5.2 Hz, OH-3′); 5.10 (1 H, d, $J$ = 5.3 Hz, OH-4′); 4.60 (1 H, d, $J$ = 5.8 Hz, OH-6′); $^{13}$C NMR [600 MHz, DMSO-d6, δ (ppm)]: δ 161.2 (C-2); 92.9 (C-3); 166.5 (C-4); 113.7 (C-4a); 137.0 (C-5); 127.7 (C-6); 131.9 (C-7); 114.8 (C-8); 154.2 (C-8a); 23.0 (C-9), 99.5 (C-1′), 73.4 (C-2′); 76.5 (C-3′); 69.4 (C-4′); 77.3 (C-5′); 60.5 (C-6′).

The compound isolated is a whitish needle-like crystal with melting point of 149 °C. Structural elucidation of $^1$H NMR and $^{13}$C NMR spectra revealed that the isolated compound is 5-methylcoumarin-4-β-glucoside (Fig. 1). The TLC analysis of the isolated compound showed a single spot with $R_f$ value of 4.5 using a solvent system comprising of hexane, ethyl acetate methanol in the ration 7:2:1. The single spot indicates the purity of the compound which was further confirmed by the clean NMR spectra. The structure of isolated compound was determined through the interpretation of $^1$H and $^{13}$C NMR data. The $^1$H NMR spectrum (Supplementary Figure S1) displayed characteristic signals of a glycoside derivative of 4-hydroxy coumarin. The two doublets at δ 7.16 ($J$ = 7.6 Hz) and δ 7.24 ppm ($J$ = 8.4 Hz) as well as the triplet at δ 7.51 ppm ($J$ = 7.8 Hz) integrated for single proton each were assigned to the aromatic protons at positions 6, 8 and 7 of the coumarin ring, respectively. Two singlets at δ 2.71 (3 H) and δ 6.0 ppm (1 H) were assigned to $CH_3$-9 and H-3, respectively. The signals of the sugar proton were observed between δ 3.2 and δ 5.2 ppm. The coupling constants of the sugar moiety protons as well as the $^{13}$C NMR signals suggested the presence of a β-D-glucopyranose. The $^{13}$C NMR showed the presence three aromatic 16 carbon atoms. The two signals downfield at δ 161.2 ppm and with d with the carbonyl signal of C-1 found down field at δ 161.2 ppm and δ 161.2 ppm were assigned to carbonyl carbon (C1) and C-4 respectively. Six aromatic signals appeared between δ 114.8 and δ 154.2 while the signal of the methyl carbon was apparent upfield at δ 23.0 ppm. Based on the interpretation of the spectroscopic data and, in comparison with previously reported literature [1,4] the isolated compound was explicitly elucidated as 5-methylcoumarin-4-β-glucoside (Fig. 1). The isolated 5-methylcoumarin-4-β-glucoside is a pure compound (~ 99.9%).

### 3.2. Acute toxicity test

During the first and second phases of the acute toxicity study, oral administration of 5-methylcoumarin-4-β-glucoside (10–5000 mg/kg) for 24 h did not produce any visible sign of toxicity or mortality in the animals. The oral $LD_{50}$ for 5-methylcoumarin-4-β-glucoside in mice was determined to be above 5000 mg/kg.

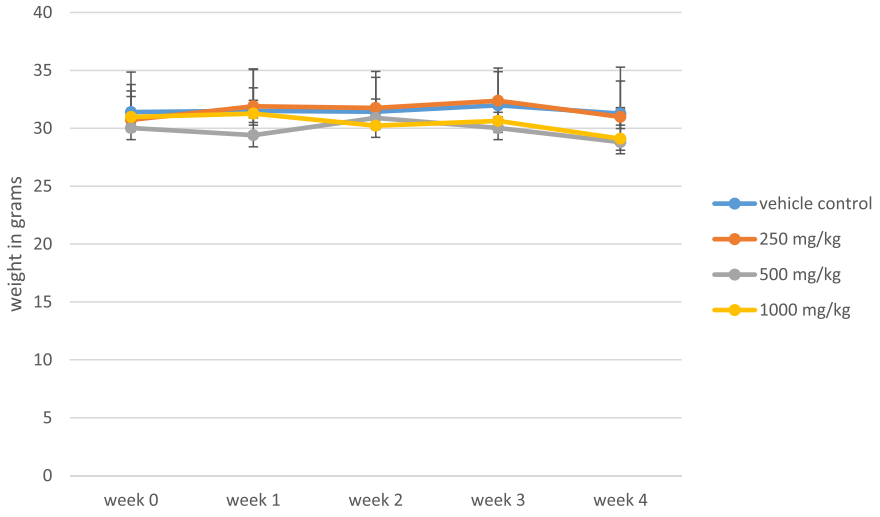


**Fig. 2.** Weight changes in mice following oral administrattion of graded doses of *5-methylcoumarin-4-β-glucoside*.

**Table 2**
Serum biochemical parameters in mice following oral administrattion of graded doses of *5-methylcoumarin-4-β-glucoside*.

| Intervention<br>*Biochemical parameter* | Vehicle control | 250 mg/kg | 500 mg/kg | 1000 mg/kg |
|---|---|---|---|---|
| Total cholesterol (mg/dL) | 127.60 ± 58.7[a] | 81.17 ± 12.4[a] | 89.20 ± 7.8[a] | 92.33 ± 16.6[a] |
| Triacyl glycerol (mg/dL) | 132.00 ± 21.6[b] | 125.00 ± 10.5[b] | 128.00 ± 27.7[b] | 131.20 ± 27.1[b] |
| Total bilirubin (mg/dL) | 1.03 ± 0.2[c] | 2.20 ± 1.3[c] | 0.82 ± 0.4[c] | 1.17 ± 0.9[c] |
| Alanine aminotransferase (u/L) | 103.20 ± 8.7[de] | 105.30 ± 29.0[de] | 44.60 ± 44.9[d] | 133.20 ± 49.2[e] |
| Aspartate transaminase (u/L) | 48.40 ± 23.2 [f] | 74.00 ± 23.8 [f] | 54.60 ± 29.0 [f] | 57.60 ± 35.3 [f] |
| Alkaline phosphatase (u/L) | 60.20 ± 16.4 [g] | 55.00 ± 25.6 [g] | 35.60 ± 21.37 [g] | 49.00 ± 14.8 [g] |
| Glucose (mmol/L) | 4.90 ± 2.2 [h] | 5.70 ± 2.4 [h] | 7.92 ± 2.1 [h] | 6.50 ± 1.2 [h] |
| Potassium ion (mmol/L) | 6.44 ± 1.3[i] | 7.41 ± 1.7[i] | 7.62 ± 1.6[i] | 6.92 ± 1.2[i] |
| Urea (mmol/L) | 6.76 ± 1.0[j] | 6.68 ± 1.1[j] | 8.74 ± 0.6[k] | 7.35 ± 0.9[jk] |
| Creatinine (mg/dL) | 1.06 ± 0.3 [l] | 0.98 ± 0.3 [l] | 1.48 ± 0.4 [l] | 1.10 ± 0.3 [l] |
| Uric acid (mg/dL) | 5.70 ± 1.3 [m] | 4.68 ± 0.9 [m] | 5.68 ± 1.1 [m] | 5.620 ± 2.0 [m] |
| Calcium ion (mmol/L) | 2.08 ± 0.1 [n] | 1.96 ± 0.1 [n] | 2.2 ± 0.22 [n] | 1.91 ± 0.5 [n] |
| Phosphate ion (mmol/L) | 0.96 ± 0.1° | 1.16 ± 0.2° | 1.09 ± 0.5° | 1.39 ± 0.1° |

Data represent mean ± SD (n = 6). Rows superscripted by the same letter are not significantly different at $p < 0.05$ in Tukey's multiple comparison test.

### 3.3. Sub-acute toxicity test

There was no variation in food/water consumption across interventions. Except for one mouse in the group administered 500 mg/kg of 5-methylcoumarin-4-β-glucoside, all the mice in the groups survived the sub-acute toxicity testing. The death of the animal is not likely due to the exposure to the test compound because its autopsy revealed that its organs remained intact after death. On day 22 of the intervention, two mice in the group with the highest dose were observed to develop alopecia (Supplementary Figure S2). Other than the above-mentioned changes, general observations revealed no changes in the respiratory rate, occurrence of secretions and excretions, and autonomic activity (such as lacrimation, piloerection, pupil size, and unusual respiratory pattern), changes in skin fur, eyes and mucous membranes, gait, posture, and sensitivity to handling, as well as the absence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling), and bizarre behavior (e.g., self-mutilation, walking backwards). In comparison, there was no significant weight change among the treatment groups throughout the intervention period (Fig. 2).

Irrespective of the dose, 5-methylcoumarin-4-β-glucoside did not affect total cholesterol and triacylglycerol levels. The serum lipid panel levels were within normal concentrations for the mice. Unconjugated bilirubin levels were also unperturbed throughout the four weeks intervention with 5-methylcoumarin-4-β-glucoside. Total bilirubin levels, serum glucose, creatinine, uric acid, potassium ion, calcium ion and phosphate ion levels were all not significantly different among the different groups of mice administered graded doses of 5-methylcoumarin-4-β-glucoside (Table 2). This was also the case with aspartate transaminase and alkaline phosphatase. As the dose of the compound increases, also serum urea levels tend to increase. In comparison, the biochemical parameters examined resulting from 5-methylcoumarin-4-

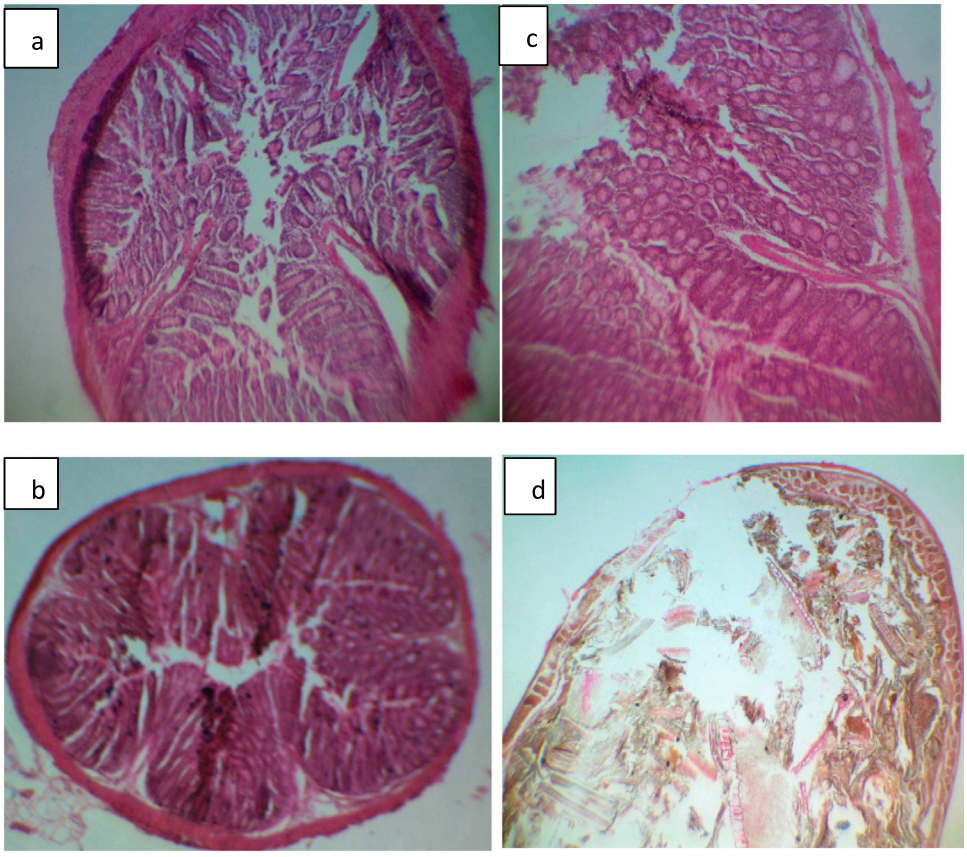


**Fig. 3.** Photomicrograph of sections of the colon of laboratory mice administered graded doses of 5-methylcoumarin-4-β-glucoside for 28 days (H and E × 100). a= vehicle control; b= 250 mg/kg; c= 500 mg/kg; d= 1000 mg/kg.

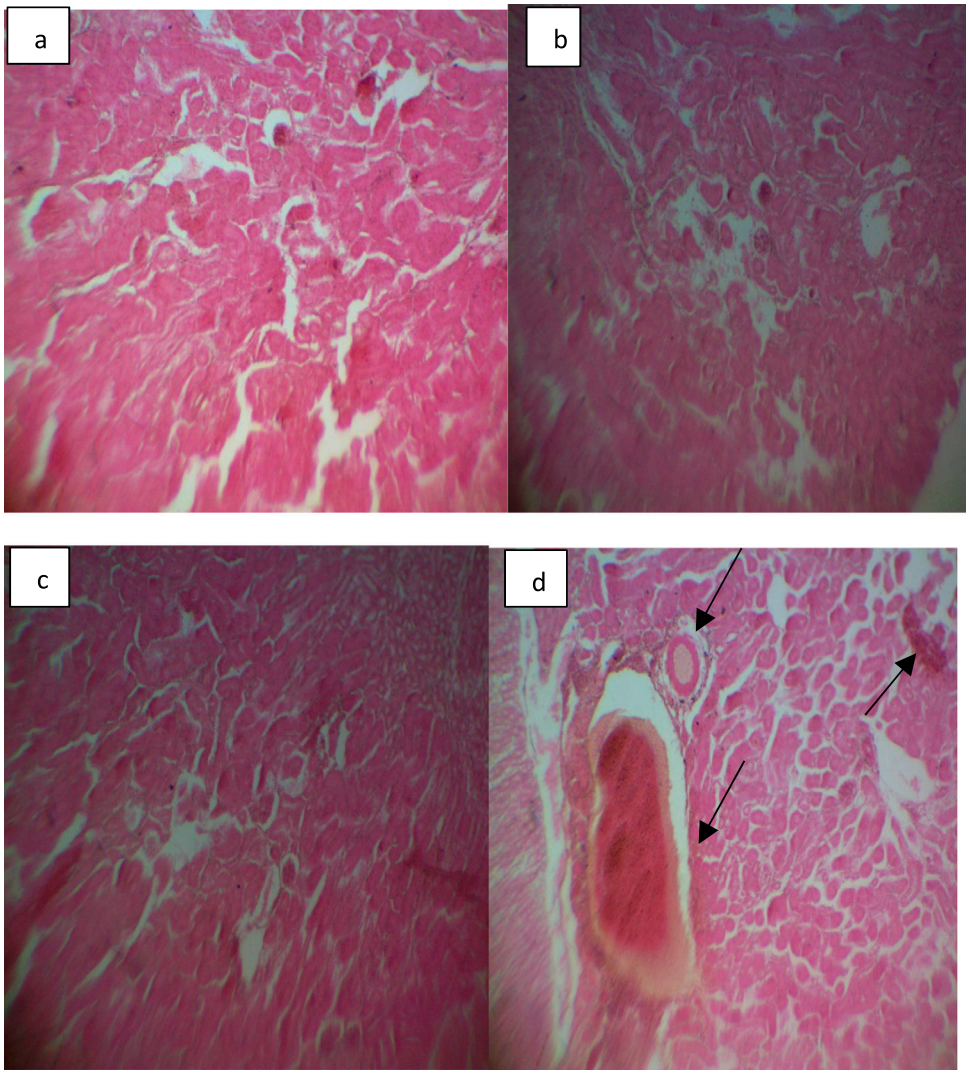


**Fig. 4.** Photomicrograph of sections of the kidney of laboratory mice administered graded doses of 5-methylcoumarin-4-β-glucoside for 28 days (H and E × 100). a= vehicle control; b= 250 mg/kg; c= 500 mg/kg; d= 1000 mg/kg. The arrows in plate d shows glomerular atrophy and congestion of interstitium and blood vessels.

β-glucoside intervention did not distort the levels of most of the serum biochemical parameters examined.

No sign of edema was recorded in the colon photomicrographs of the vehicle control (Fig. 3a) and low dose (Fig. 3b) groups of mice. The goblet cells and other mucosal and muscular layer features were normal. Fig. 3c and d show a dose dependent progression in crypt destruction, total depletion of goblet cells and mucosa erosion. The group that received the highest dose (Fig. 3d) showed the least intact colonic mucosa with a depleted epithelium structure.

Except for the high dose group, histology of the kidney showed no signs of glomerular atrophy or congestions of interstitium and blood vessels (Fig. 4). These features were present in the high dose of 5-methylcoumarin-4-β-glucoside. The photomicrograph showed no sign of necrosis in all the groups administered 5-methylcoumarin-4-β-glucoside.

Fig. 5a through d show a progression in the distortion of the hepatic architecture of the liver tissues of mice administered graded doses of 5-methylcoumarin-4-β-glucoside (Fig. 5). The group administered 500 mg/kg of 5-methylcoumarin-4-β-glucoside demonstrates congestion of portal vein. The group administered 1000 mg/kg of 5-methylcoumarin-4-β-glucoside showed necrotic foci filled with edema. Histopathological examination of hepatic, colon and kidney tissues of mice administered 1000 mg/kg of 5-methylcoumarin-4-β-glucoside exhibited the most histological deformation compared to the other doses. However, they were not exhibited in serum biochemical parameters.

## 4. Discussion

5-methylcoumarin-4-β-glucoside is a promising anticancer agent against colon cancer disease [1]. It was isolated from the leaves of *Vernonia glaberrima* and characterized as a coumarin derivative. Many derivatives of coumarin have been demonstrated to exhibit anticancer activity via acting as cyclin-dependent kinase-specific ATP-competitive inhibitors [9]. More importantly, coumarin derivatives with substitution at position 4 have been shown to demonstrate antiproliferative activity against breast [10] and liver carcinomas [2]. Similar to the aforementioned coumarin derivatives, 5-methylcoumarin-4-β-glucoside also has a sugar substitute at position 4 (Fig. 1), which might be partly contributory to its *in vitro* anticancer activity against colon cancer (HT-29) cell lines [1]. This is in addition to the anticancer effect of the parent moiety (2 H-1-benzopyran-2-one).

Evaluation of the toxicity profile of a promising therapeutic agent is unarguably at the forefront of any drug discovery process. Acute toxicity

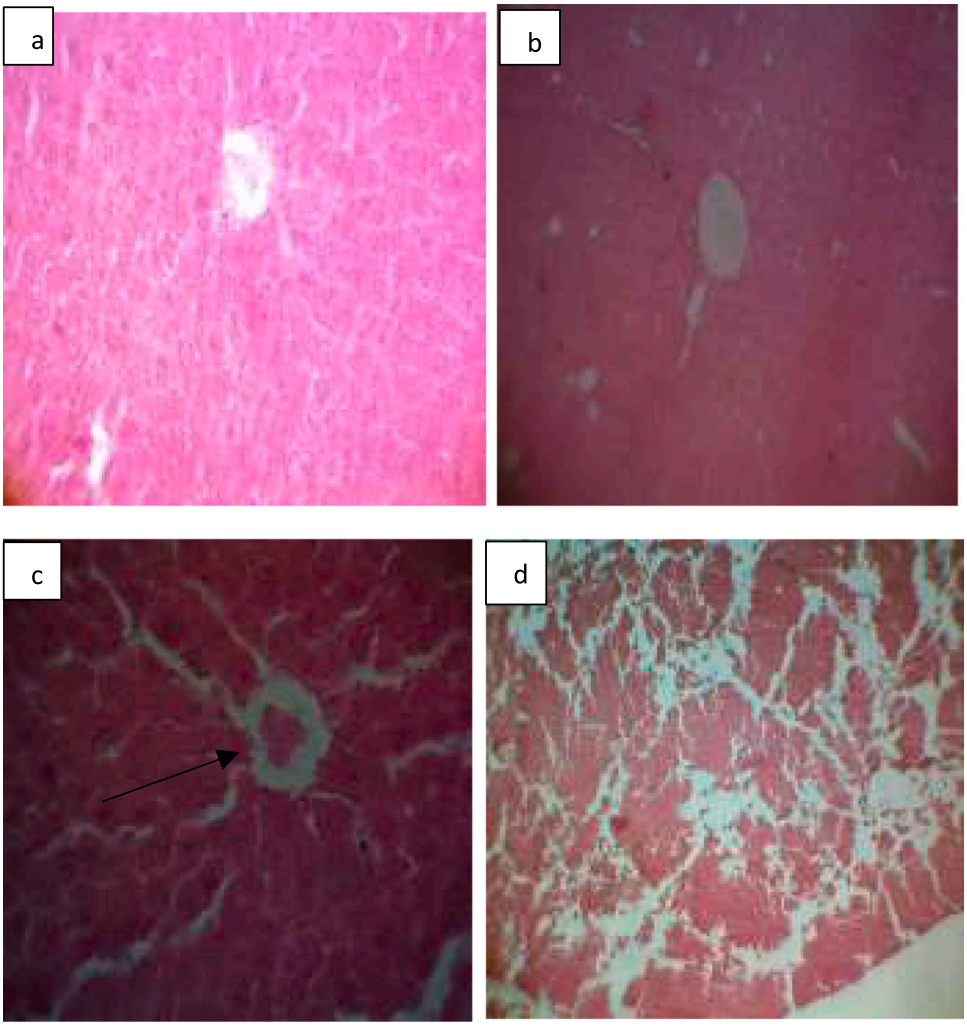


**Fig. 5.** Photomicrograph of sections of the liver of laboratory mice administered graded doses of 5-methylcoumarin-4-β-glucoside for 28 days (H and E × 100). a= vehicle control; b= 250 mg/kg; c= 500 mg/kg; d= 1000 mg/kg. The arrow in plate c shows slight congestion of portal vein.

study is usually the first type of toxicity study investigated *in vivo* during preclinical drug development. It is aimed at providing a guide for the dose range of a novel therapeutic agent and provides preliminary information on the mode of toxic action of the agent in question [8]. 5-methylcoumarin-4-β-glucoside exhibited an $LD_{50}$ of over 5000 mg/kg body weight. This provides a broadband of dosage selection and optimization for further therapeutic and toxicity studies. It also gives a preliminary insight into the short term safety of the compound. Weight loss could be challenging during many active cancer diseases, treatment with an anticancer agent that causes weight loss could be further debilitating. Irrespective of the dose administered, 5-methylcoumarin-4-β-glucoside did not affect the weight of the mice during the 28-day sub-acute toxicity study.

The mortality observed in the group that was administered 500 mg/kg bodyweight of the compound was unlikely due to the compound. This is because no similar mortality was observed in the group that received a higher dose (1000 mg/kg). 5-methylcoumarin-4-β-glucoside did not alter serum glucose and electrolyte levels. It did not also alter the liver enzymes and serum lipids investigated. The serum urea level is the only serum biochemical parameter that differed among the groups. In comparison, findings from serum biochemical parameters showed no difference among the groups of mice. In contrast, histology of the analysed tissues showed evident graded dose-dependent distortion of the cytoskeleton in the colon, kidney and liver in a graded dose-dependent manner. Serum biochemical parameters usually corroborate histology findings. In this scenario, that was not the case. All of the serum biochemical parameters related to the liver and kidney (except for urea) did not reflect the exhibited histopathological findings in these organs. This could be so because these biochemical parameters are products of degenerated structure (histology) and functions of cells and tissues of the organ in question. Put more clearly, degeneration of the structure of an organ precedes its altered biochemical marker.

The upper limit dose at which 5-methylcoumarin-4-β-glucoside exhibited its histological distortion (1000 mg/kg) is quite high. This is an advantage in safety terms for the candidate anti-colorectal cancer agent as pilot studies have shown that its expected therapeutic dosage range is quite lower than the dose that distorted the histology of the organs. Taking all together, 5-methylcoumarin-4-β-glucoside demonstrated minimal histopathological toxicity at the highest dose and almost no toxicity in sero-biochemical terms after a 28-day toxicity study.

## 5. Conclusion

Based on the acute toxicity study, the $LD_{50}$ of 5-methylcoumarin-4-β-glucoside was determined to be greater than 5000 mg/kg. The 28-day sub-acute toxicity study revealed that the compound is well tolerated up to a dose of 500 mg/kg. This study therefore demonstrates the relative tolerability of 5-methylcoumarin-4-β-glucoside up to 500 mg/kg. However, since this is a preliminary toxicological study, a more

comprehensive toxicity study involving non-rodent animals is required to corroborate our findings and suggest safe dose levels for clinical studies.

## CRediT authorship contribution statement

**Bilyaminu Abubakar**: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Supervision, Project administration, Funding acquisition. **Alhassan Muhammad Alhassan**: Conceptualization, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Ibrahim Malami**: Validation, Formal analysis, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Dawoud Usman**: Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization. **Yaaqub Abiodun Uthman**: Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization. **Kehinde Ahmad Adeshina**: Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization. **Mutolib Olatubosun**: Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization. **Mustapha Umar Imam**: Validation, Formal analysis, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2022.03.013.

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