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Research article

Sub-acute and sub-chronic toxicity assessment of the antimicrobial peptide Dermaseptin B2 on biochemical, haematological and histopathological parameters in BALB/c mice and Albino Wistar rats

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ABSTRACT

Background: Dermaseptins (Drs) are peptides found in the skin secretions of a variety of Hylid frogs, particularly those belonging to the Agalychnis and *Phyllomedusa* families. Dermaseptin B2 (Drs B2), an amphipathic, α-helical polypeptide was reported as the most active of the Dermaseptin B family. We have previously shown that Drs B2 has strong anti-proliferative activities against RD cells *in vitro* and thus required further evaluations for future medical applications.

Aim: The aim the study was to evaluate the 14-day sub-acute and 90-day sub-chronic toxicities Drs B2 *in vivo*.

Materials and Methods: BALB/c mice were treated with increasing concentrations of 5–25 mg/kg of Drs B2. Rats were treated with 2, 4 and 10-fold concentrations of the calculated LD_{50} of Drs B2 following OECD recommendations. At the end of the experimentation periods, the animals were sacrificed and dissected to collect blood and selected organs for analysis of any effects caused by Drs B2 treatment on the biochemical, haematological, and histological parameters.

Results: The 14-day sub-acute toxicity tests did not cause significant alteration in the biochemical, hematological and histological parameters. The 90-day sub-chronic toxicity study showed lower ALT and AST than control at doses 1.9 mg/kg and 4.6 mg/kg, respectively. Their haematology results also showed higher platelet count than the controls but the differences were not statistically significant. Histological analysis showed increased megakaryocytes in the spleen for both the mice and the rats.

Conclusion: The results of this study indicate that short term treatment of Drs B2 could be safe to the animals, however, long-term treatment can have mild effects on the liver parameters and cause an inflammatory response in the spleen.

1. Introduction

Antimicrobial peptides (AMPs) play a significant role in the natural immunity of different organisms like animals, insects and amphibians [1, 2]. These peptides possess an amphipathic structure [3], and interact with the negatively charged molecules on the cell membranes [4, 5]. Interaction of AMPs with the membrane leads to its disruption [5], inducing intracellular components to leak and cell death [6, 7]. The mode of action of these peptides is not mediated through definite receptors thus less affected by resistance development [8]. Antimicrobial peptides bind to host components including extracellular proteins and membrane lipids as well as anionic components which can affect their bioavailability [9, 10]. Despite the fact that various antimicrobial peptides have been isolated and described, there have been little research on toxicity studies [11], and studies that show their implications and their use for the treatment of systemic infections, assessment of their *in vivo* safety is also imperative [12].

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In comparison to traditional antibiotics, antimicrobial peptides (AMP) have the potential for broad-spectrum activity, quick bactericidal activity, and a low tendency for resistance development. More than 600 AMPs derived from various organisms have been discovered to date, and they not only kill pathogenic microorganisms, but also play an important role in recruiting and promoting elements of the innate immune system and have been extensively tested in *in vitro* anticancer studies with promising activities [13]. Since their antimicrobial mechanisms are not fully known, few investigations on the short-term and long-term effects of AMPs have been published.

The family of dermaseptins (Drs) peptides are derived from the skin secretions of the arboreal South American frogs [14, 15, 16]. They are short 24–34 residue peptides with net positive charge [17, 18]. Dermaseptin B2 also called adenoregulin [19], an amphipathic, α-helical polypeptide [20], was reported as the most active of the Dermaseptin B family [17], and is isolated from *Phyllomedusa bicolor* [16, 21]. It was reported to exhibit potent membranolytic activity against diverse micro-organisms with less hemolytic activity [22, 23]. We have previously shown that Drs B2 has strong anti-proliferative activities *in vitro* and thus required further evaluations for future medical applications [24]. In the current study, we investigated the sub-acute and sub-chronic toxicity of the antimicrobial peptide Drs B2 in BABL/c mice and Wistar rats, because toxicity is a key factor in the development of antimicrobial peptides as medicinal agents. In addition, physical behavior and body weight changes were observed in mice and rats. We also looked at how Drs B2 affected hematological parameters as well as plasma enzymes, all of which are crucial indicators of toxicity in medical treatment and their side effects.

2. Materials and methods

2.1. Dermaseptin B2

The antimicrobial peptide Dermaseptin B2 (Adenoregulin, lot no: 2020/10/14) was synthesized by Solar Bio (Beijing, China). The peptide was dissolved in sterile phosphate buffered saline (PBS). The study was approved and validated by the PAUSTI board of examiners (MB400-0007/2019).

2.2. Animals

Male BALB/c mice were bred at the Small Animal Facility for Research and Innovation (SAFARI) laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT). Animals were randomly grouped in specific plastic cages with unique identification numbers. Animals were acclimatized for one week before experimentation under pathogen-free conditions, free access to water and animal feed and 12h/12h light/dark cycling.

2.3. Ethical statement

All the animal studies were carried out according to the Institutional Animal Care and Use Committee approved by Institutional Scientific and Ethics Review Committee (ISERC) (Ref. ISERC/07/2021) Institute of Primate Research (IPR), National Museums of Kenya. All animal procures were carried out in a manner that minimized the number and suffering of the animals used in the study.

2.4. Animal treatments

2.4.1. Acute toxicity study

This study was designed according to the OECD guideline for testing of chemicals [25]. This protocol was carried out to evaluate early toxicity related signs after treatment with Dermaseptin B2. Six male BALB/c mice (24–26g) were grouped into 6 groups with one mouse per group. A dose range of 5, 10, 15, 20, 25 mg/kg was injected via intra-peritoneal route (IP, 0.1ml). One mouse was used as a control and received only PBS (0.1ml) to observe if there was any toxicity related to the solvent. Animals were observed for the first 30-minutes followed by 4-hour observations for up to 24 h, then continuously for five days. Based on the results of this preliminary tests, a 14-day toxicity study was carried out.

2.4.2. Sub-acute toxicity tests

This study was designed according to the OECD guideline for testing of chemicals [25]. Twenty-five male BALB/c mice (24–33g) were grouped into five groups of five mice per group ($n = 5$). The 90-day treatment groups received 5, 10, 15 and 20 mg/kg and the control group received 0.2 ml PBS solvent only. Animals were subjected to the treatment daily for 14-days via an IP route. Food and water intake were checked on continuous basis and the body weight was recorded on day 1, 7 and 14. Any signs related to toxicity including changes in animals' behavior, skin fur, motility, response to stimulation and activity were recorded. Death of two animals was recorded at doses 15 mg/kg and 20 mg/kg on the 9^{th} and 13^{th} day of the treatment, respectively. An LD_{50} was then calculated using Karber's method. Animals were sacrificed using CO_2 asphyxiation and samples including blood and organ tissues were collected. Tissues were fixed in 4% formaldehyde and replaced after 24 h.

2.4.3. Sub-chronic toxicity tests

A 90-day sub-chronic toxicity was carried out according to the OECD 90-day toxicity test guideline [26]. Twenty-four male Wistar rats were grouped into four groups (6 rats/group) comprising three treatment groups and one control group. The treatment groups received two, four and ten-folds of the LD_{50} (18.5 mg/kg) of 1.9, 4.6, and 9.3 mg/kg. The control group received only PBS (solvent). Both the test and control group received a volume of 10 ml/kg. Animals were observed daily for general appearance, changes in behavior, morbidity or mortality. Food consumption and water intake were observed on daily basis. After 24-hours of the last treatment, animals were sacrificed and samples were collected.

2.5. Biochemical parameters

Blood samples were collected by cardiac puncture in plain vacutainer tubes and serum was obtained by centrifugation at 4, 000 rpm for 10 min at 4°C. At the end of the experiments, blood parameters including alanine aminotransferase (ALT), aspartate amino transferase (AST), creatinine (Cr) and blood urea nitrogen (BUN) were analyzed using Refletron machine (Roche, Germany).

2.6. Hematological parameters

Routine hematology parameters were analyzed with Mindray automated hematology analyzer (ROCHE, Germany). Blood samples were collected in EDTA-K2 tubes for the hematological analysis at the end of the experiments. The following parameters were analyzed red blood cells (RBC), hemoglobin (Hb), white blood cell count (WBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hematocrit (MCH), mean corpuscular hemoglobin concentration (MCHC), Lymphocytes, and Platelet count (PLT).

2.7. Gross necropsy

At the end of the experiments, treated and control animals were euthanized and necropsied using CO2 asphyxiation. Necropsies were performed for animals which died or survived during experimentation. Tissues including liver, kidney, heart, spleen and lungs were macroscopically examined for general morphology. Tissues were kept in 4% paraformaldehyde which was replaced after 24-hours. The relative weight of the organs (organ weight/body weight ratio) was calculated based on the animal using the formula: organ weight/body weight on the day of sacrifice x 100 [27].

2.8. Organ histopathological examination

Tissues were dehydrated in ethyl alcohol, embedded in paraffin and sectioned at **5-μm** thickness. Sections were stained with hematoxylin and eosin (H&E) and examined under the microscope (40x magnification). Results were confirmed by experienced Histopathologist.

2.9. Statistical analysis

Data were expressed as mean and standard error of mean (mean ± SE). All statistical analysis of the body weight (BTW), organ weight, hematological and biochemical parameters were analyzed using one-way analysis of variance (ANOVA) using multiple comparison tests to separate the variant means' (Tukey test) in GraphPad Prism Software (GraphPad Prism, San Diego, USA). P value < 0.05 was considered as statistically significant between the treatment and control groups.

3. Results

3.1. Acute toxicity tests

Treatment of BALB/c mice with Dermaseptin B2 (Drs B2) showed changes in the behaviors of the mice during the first 4-hours. Mild signs of toxicity including fur changes, isolation and grooming were observed at a dose of 25 mg/kg. The mice at this latter dose died after 72-hours of treatment. Mild signs of toxicity including fur changes, grooming and hypo-activity were observed at 20 mg/kg dose. These signs disappeared within the first 4 h. No toxicity related signs were observed at doses of 5, 10, 15 mg/kg. The results indicate that Drs B2 did not influence routine behaviors of the animals at doses less or equal to 15 mg/kg. Gross pathology assessment did not show any observable pathologies induced by the peptide.

3.2. Sub-acute toxicity tests

Sub-acute toxicity study was assessed by single daily injection of Drs B2 via an I.P route for 14-days to groups of BALB/c mice (n = 5 mice/group) and LD_{50} (18.5 mg/kg) was determined. No changes in the behavior or mortalities were recorded for doses of up to 10 mg/kg. Mild fur changes and stress were observed for the 20 mg/kg dose throughout the treatment period. Death of two animals was recorded at doses of 15 mg/kg and 20 mg/kg on the 9^{th} and 13^{th} day, respectively. Gross examination of the organs did not reveal any observable side effects.

3.3. Sub-chronic toxicity studies

The 90-day sub-chronic toxicity test was carried out using male Wister rats to evaluate the long-term effects of Drs B2 by an I.P injection of 1.9, 4.6, and 9.3 mg/kg. No treatment related deaths were registered in the treatment groups over the period of the treatment.

3.4. Food and water intake and body weight

Changes in food consumption and water intake were monitored daily but no changes were observed for sub-acute and sub-chronic tests, indicating that Drs B2 did not affect food consumption and water intake. There were no significant differences ($p > 0.05$) between the body weight of the test and the controls groups except slight significant increase on the 7^{th} day of the 20 mg/kg group ($p^{<} 0.05$). No significant differences were found between the treatment groups and the control group. The body weights of mice treated with Drs B2 for the 14-day study are presented in Figure 1. Likewise, the 90-day treatment of rats with Drs B2 did not lead to any difference in weight between the groups. The routine behaviors were normal including food consumption, movement and excretion.

3.5. Effect of drs B2 on relative organ weight

During the treatment period, the relative organ/body weight ratio (organ weight/body weight x 100) was not significantly ($p^{>} 0.05$) different between the control and the treatment groups (Figure 2). No treatment related macroscopic examination were identified in the treatment groups. The relative organ weight of the rats treated with Drs B2 for the chronic tests is presented in Figure 3.

3.6. Biochemical tests

Biochemical parameters including ALT, AST, creatinine and Urea were analyzed in blood samples. Table 1 shows levels of biochemical parameters after treatment of BALB/c mice with Drs B2. Using ANOVA (Tukey multiple comparison test), these results did not show any dose-related significant differences between the control and the treatment groups for the liver and kidney parameters.

Table 2 shows the results for the organ function tests of the 90-day sub-chronic toxicity test. Doses of 1.9 mg/kg and 4.6 mg/kg showed lower ALT values than the control but when these results were compared using ANOVA test (Tukey's test), it did not lead to any significant differences ($p^{>}0.05$) between the treatment and the control group. There was lower AST value at 1.9 mg/kg than the control but ANOVA results did not show significant differences ($p^{>}0.05$) between the control and this dose group. There were no dose-related differences between the creatinine and Urea results when compared to the control group.

3.7. Hematological tests

There were no significant dose-related differences ($p > 0.05$) were found in all the hematological parameters between the control and the treatment groups except the Platelet count (PLT) which was significantly ($p < 0.05$) lower than the control group at a dose 10 mg/kg (Table 3). No significant differences were found between the treatment groups.

The hematology results for the 90-day treatment of Drs B2 with rats are shown in Table 4. The results indicate no significant differences ($p^{>}0.05$) between the treatment groups and the control with the exception of plate count which was slightly higher than the control at dose 1.9 mg/kg, but the differences were not significant ($p^{>}0.05$).

3.8. Gross necropsy and histopathological tests

Macroscopic examination of the organs showed no dose-dependent changes or lesions in the general appearance and the architecture of the vital organs. No inflammation, hypertrophy, edema, or atrophy were observed on the organs. The histopathological examination of liver, kidneys, heart, did not show any pathological change. There were increased megakaryocytes and multinucleated cells in the spleen Figure 4 although the normal architecture was intact and no changes in the follicles was observed. Similarly, there was no degeneration or any inflammatory or necrotic effects seen as shown in (Figure 4).

Figure 5 represents the 90-day treatment histology results for the rats treated with Drs B2. The histopathological examination of liver, kidneys, heart, did not show any pathological change. There were increased multinucleated cells in the spleen which could be an inflammatory response due to treatment of Drs B2. No changes were observed in the follicles, and neither degeneration nor necrotic effects were seen.

4. Discussion

Dermaseptins are a broad class of antimicrobial peptides found in amphibian skin. In polar solvents, these linear polycationic peptides are unstructured, but in a polar solvent, they rapidly transition to an amphipathic α-helix. Dermaseptins have cytolytic activity against a wide range of non-host microorganisms *in vitro*, including bacteria, protozoa, yeasts, and filamentous fungi [28]. Cationic antimicrobial peptides have

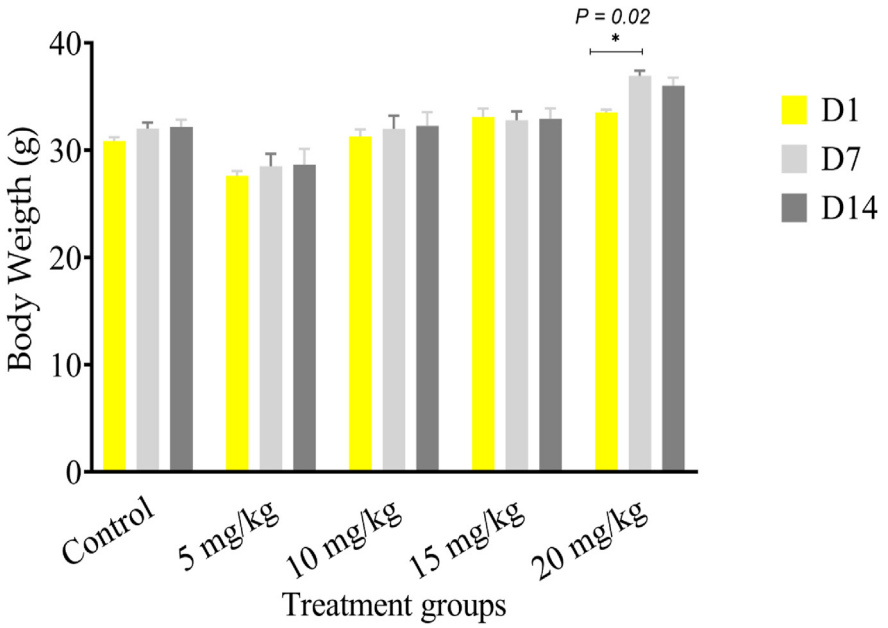

Figure 1. Body weights of the BALB/c mice groups (g) treated with Drs B2 over a 14-day period.

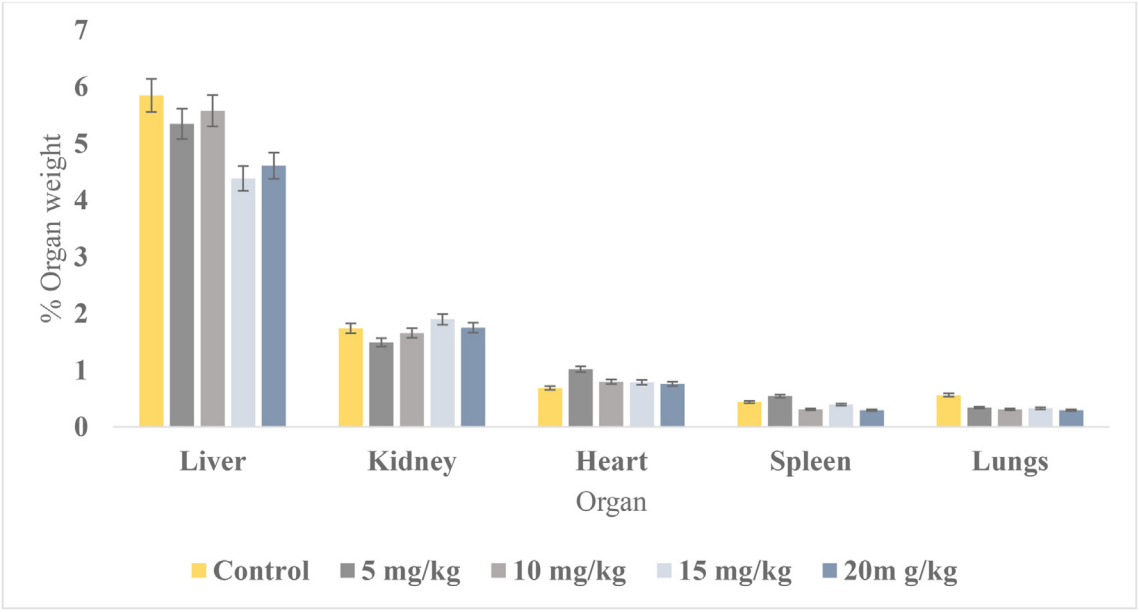

Figure 2. The % relative organ weight (% relative organ to body weight) for BALB/c mice treated with Drs B2.

gained the interest of both the scientific community and the pharmaceutical industry in recent years due to their potential as new therapeutic agents. However, it is usually assumed that this class of drugs lacks specificity and may be too toxic for systemic use [28]. As a result, numerous antimicrobial peptides that are now undergoing clinical studies have been chosen for topical application [29].

In this study, we investigated the sub-acute and sub-chronic toxicity of the antimicrobial peptide Drs B2 in BABL/c mice and Wistar rats, as toxicity tests represent a key factor in the development of antimicrobial peptides as medicinal agents. We also evaluated at how Drs B2 affects on the hematological parameters as well as plasma enzymes, all of which are crucial indicators of toxicity in medical treatment and their side effects.

Administration of BALB/c mice with Dermaseptin B2 (Drs B2) adverse effects, although changes in the behaviors of the mice during the first 30 min but these signs resolved in 4-hours post treatment. On the other hand, mild signs of toxicity including fur changes, isolation and grooming were observed at a dose of 25 mg/kg for the acute tests. Mortality of the mice 25 mg/kg was after 72-hours, this result indicates that doses beyond 20 mg/kg could induce adverse effects on the animals. Results of the 14-day toxicity study revealed no significant effects of the peptide on normal weight and routine behaviors of mice, although mild signs of toxicity were observed including mild fur changes and stress, and lead to the death of two animals during the 14-day toxicity study at doses 15 mg/kg and 20 mg/kg, respectively. In line with our results, a study using three derivatives of dermaseptin S4 peptide reported an *in vivo* acute toxicity of these peptides after an I.P. injection of a single dosage of each peptide into groups of BALB/c mice. Mild fur erections were reported for doses greater than 5.4 mg/kg minutes after injection and resolved in all cases in 4 h or less [28]. The antimicrobial peptide S-thanatin was also reported to cause no adverse side effects and did not affect the normal behavior of mice in dosages of up to 125 mg/kg [13]. It was also reported that SET-M33L was less toxic up doses of 20 mg/kg

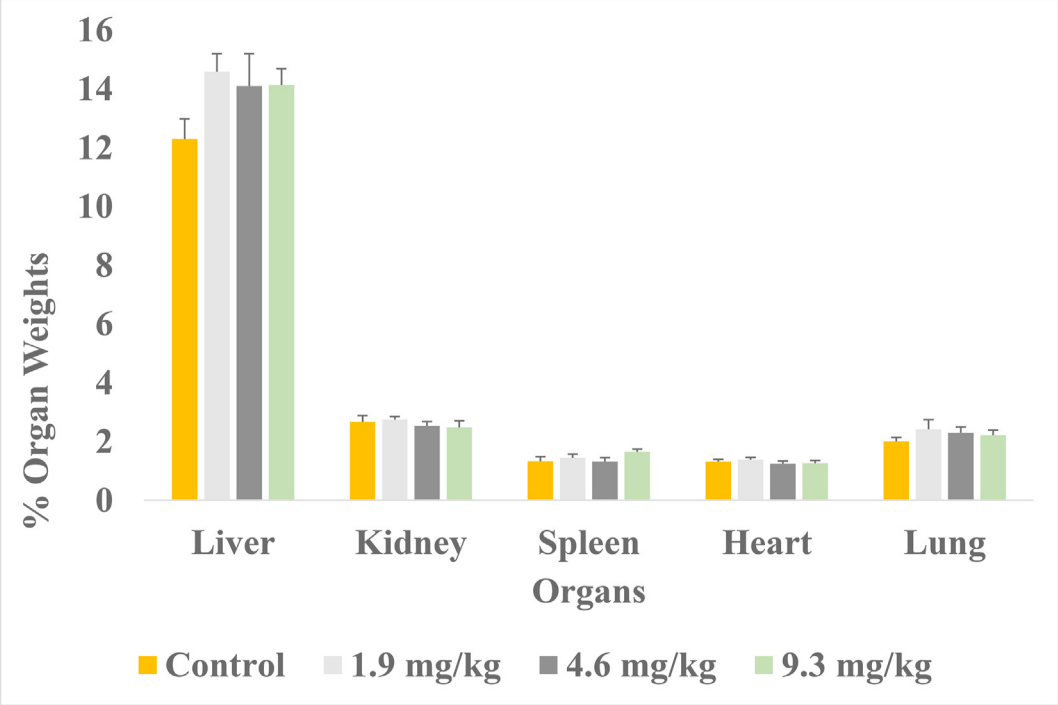

Figure 3. The % relative organ weight of the rats treated with Drs B2 (% organ body weight ratio).

Table 1. Organ function tests for mice after 14-day treatment with Drs B2.

Dose (mg/kg Body weight)- 5 mice/group	Parameter			
	ALT (IU/L)	AST (IU/L)	Creatinine (mg/dL)	Urea (mg/dL)
Control	39.26 ± 4.33	33.82 ± 4.12	0.48 ± 0.01	21.8 ± 0.30
5 mg/kg	34.02 ± 1.94	25.52 ± 1.86	0.47 ± 0.01	21.48 ± 0.20
10 mg/kg	29.26 ± 3.20	31 ± 2.81	0.48 ± 0.01	22.42 ± 0.51
15 mg/kg	33.86 ± 3.95	30.16 ± 2.68	0.46 ± 0.01	24.94 ± 2.94
20 mg/kg	31.36 ± 1.34	30.88 ± 3.23	0.48 ± 0.01	21.96 ± 0.49

Table 2. Organ function tests for the 90-day sub-chronic toxicity study for the rats treated with Drs B2.

Dose (mg/kg Body weight)	Parameter analyzed			
	ALT (IU/L)	AST (IU/L)	Creatinine (mg/dL)	Urea (mg/dL)
Control	70.10 ± 2.86	153.17 ± 12.47	0.50 ± 0.00	44.73 ± 1.25
1.9 mg/kg	45.85 ± 7.63	92.62 ± 18.93	0.50 ± 0.00	40.57 ± 2.61
4.6 mg/kg	50.93 ± 5.33	163.35 ± 32.16	0.53 ± 0.02	37.43 ± 3.52
9.3 mg/kg	88.47 ± 12.34	207.33 ± 19.95	0.50 ± 0.00	47.33 ± 1.80

than colistin (a peptide antibiotic used in clinical practice) which produced strong signs of toxicity at dose 10 mg/kg in a 4-day toxicity study [30]. The 90-day sub-chronic toxicity study did not show changes in the behavior and or mortalities in the rats administered with Drs B2. *In vivo* sub-chronic toxicity investigation of the antimicrobial peptide P34 in

Table 3. Hematological results for the 14-day acute toxicity of BALB/c mice treated with Drs B2.

Parameter	Control	5 mg/kg	10 mg/kg	15 mg/kg	20 mg/kg
RBC (1012/L)	7.74 ± 1.34	8.30 ± 1.75	9.85 ± 0.56	9.68 ± 0.48	9.628 ± 2.34
Hb (g/dL)	11.64 ± 1.95	12.34 ± 2.87	14.98 ± 0.90	14.76 ± 0.99	14.82 ± 3.45
HCT (%)	36.54 ± 4.89	39.94 ± 8.59	46.02 ± 2.87	44.28 ± 2.89	45.88 ± 9.71
MCV (fl)	47.54 ± 2.32	48.14 ± 1.47	46.74 ± 1.71	45.72 ± 1.31	47.78 ± 2.74
MCH (pg)	15.06 ± 0.32	14.82 ± 0.47	15.24 ± 0.39	15.22 ± 0.46	15.36 ± 0.36
MCHC (g/dL)	31.74 ± 1.13	30.82 ± 0.84	32.6 ± 0.44	33.26 ± 0.13	32.14 ± 1.11
WBC (109/L)	13.48 ± 9.05	8.69 ± 1.22	8.06 ± 1.68	14.71 ± 5.93	8.602 ± 3.82
NEU (109/L)	6.82 ± 6.42	2.24 ± 0.58	1.714 ± 0.69	5.08 ± 3.87	2.224 ± 1.31
LYM (109/L)	6.62 ± 2.81	6.45 ± 1.41	6.34 ± 1.70	9.62 ± 3.46	5.978 ± 2.07
PLT (109/L)	745.6 ± 457.90	798 ± 567.10	**367.2 ± 266.5***	569.40 ± 493.6	891.4 ± 457.80

*Significantly different from the control ($p < 0.05$).

mice was also showed no death and or resulted in significant changes in body weight growth across groups [11]. The results of peptide acute toxicity testing, along with *in vivo* experiments of therapeutic and dose-response activity, suggested that the selectivity of these peptides further enhances the acceptability in the clinical practice [30].

Following the biochemical analysis of serum aminotransferases (ALT and AST), Creatinine and Urea levels of the experimental group did not reveal adverse effects of Drs B2 in contrast to the control group, and no evident dose-dependent association was seen. These findings imply that Drs B2 did not show any adverse side effect on the biochemical activities of BALB/c mice. Similar results were reported in an IRC mice treated with S-

Table 4. Hematology results of the 90-day sub-chronic toxicity study of the rats treated with Drs B2.

Parameter	Control	1.9 mg/kg	4.6 mg/kg	9.3 mg/kg
RBC (1012/L)	8.24 ± 0.43	7.24 ± 1.09	6.41 ± 1.11	6.79 ± 0.44
Hb (g/dL)	13.92 ± 0.95	12.62 ± 1.89	11.25 ± 2.04	12.35 ± 0.85
HCT (%)	0.404 ± 0.02	0.36 ± 0.05	0.328 ± 0.06	0.36 ± 0.03
MCV (fl)	48.98 ± 0.58	50.650 ± 0.58	51.47 ± 0.69	53.48 ± 0.79
MCH (pg)	16.85 ± 0.30	17.47 ± 0.09	17.65 ± 0.28	18.12 ± 0.29
MCHC (g/dL)	34.37 ± 0.41	34.45 ± 0.33	34.33 ± 0.32	33.90 ± 0.21
WBC (109/L)	11.74 ± 2.28	12.02 ± 2.81	5.51 ± 1.18	8.53 ± 2.41
NEU (109/L)	2.17 ± 0.51	2.44 ± 0.59	1.30 ± 0.20	1.69 ± 0.49
LYM (109/L)	9.54 ± 1.84	9.52 ± 2.21	4.15 ± 1.05	6.81 ± 1.96
PLT (109/L)	355.33 ± 79.50	500.00 ± 134.79	208.17 ± 67.15	218.17 ± 89.47

thanatin [13]. Other acute toxicity studies also reported no adverse effects of Hybrid peptide H4 in mice [31]. On the other hand, the 90-day sub-chronic toxicity results showed low ALT values of the experimental group compared to the control at doses 1.9 mg/kg and 4.6 mg/kg, and low AST at 1.9 mg/kg dosage compared to the control group. Although these biochemical parameters were found to be lower in the biochemical test results than in the control group, the changes were not statistically significant (p˃0.05), and no clear dose-dependent association was seen. Administration of Drs B2 suggest no adverse side effects on the physiological and biochemical functioning of the animals. The result might not indicate or raise concerns that the peptide induces liver injury as low levels of serum enzymes is not concerning, and may occasionally be seen in toxicological studies; and an etiology for these changes is often undetermined. In general terms, however, low levels of ALT and AST might be suggestive decreased production or release, a loss of functional hepatic mass, inhibition of enzyme activity, or inhibition of the coenzyme pyridoxal 5′phosphate. On the other hand, gross appearance and histology of the liver did not reveal observable degeneration of the liver histology. Notwithstanding with these findings, a previous study evaluating sub-chronic toxicity of P34 peptide reported no changes in serum amino transferases while nisin peptide caused elevated levels of ALT in mice [11].

In our study, the hematological results showed that all the parameters were in the range of the control group except the platelet count at dose 10 mg/kg which was significantly lower than the control ($P < 0.01$) group, which might be associated with bleeding or can cause thrombocytopenia. The sub-chronic toxicity results also showed, higher platelet count at dose 1.9 mg/kg group than the control group though the difference was not significant (p˃0.05). An *in vivo* study assessing the toxicity and antimycotic efficacy of P19 (8) in a mouse model showed that the peptide was well tolerated intravenously up to a single dose of 12.5 mg/kg and determined that for a 5 mg/kg peptide, an injection of (30 μM) might be sufficient to produce the antimycotic effects without causing significant depletion of host immune cells, even after accounting for the peptide's likely rapid reduction in bioavailability due to binding to cells and plasma compatibles [12]. Compared to the normal WBC results in the current study, Guoqiu *at. al* (2012) reported in an ICR mice treated with the antimicrobial peptide S-thanatin to cause low WBC value compared to the control at 125 mg/kg dose, indicating destruction of WBC and affecting animal's ability to fight infections [13].

Histopathological analysis of the liver, kidney, heart, lung of the control and treated animals indicated no morphological changes in these organs due to Drs B2 treatment. In contrast to our findings, the antimicrobial peptide S-thanatin was reported to induce pathological changes in liver and lungs at doses 20 mg/kg and 50 mg/kg, respectively [13]. The histology of the spleen indicated presence of megakaryocytes in the groups

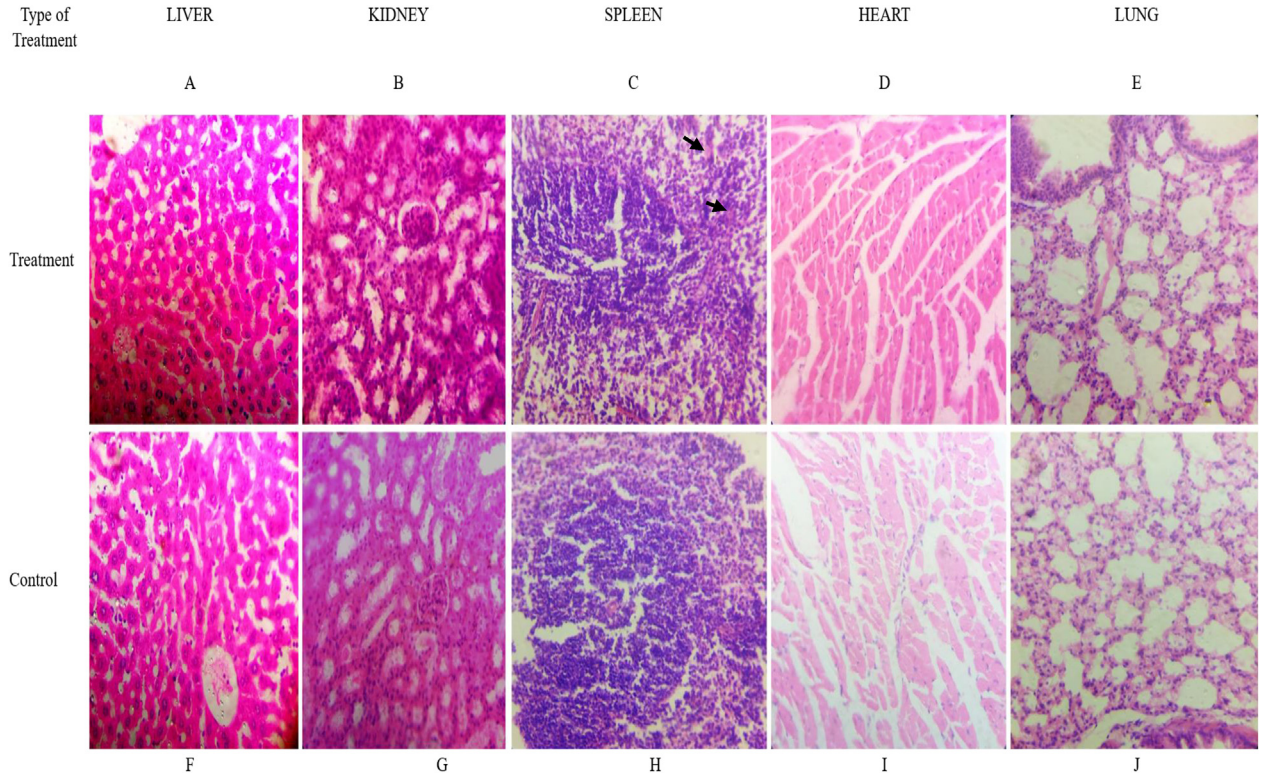

Figure 4. H&E histology Images for BALB/c mice treated with Drs B2 (5, 10, 15, 20, 25 mg/kg). A&F: liver for the test and Control mice, B&G: Kidney for the test and the control, C&H: Spleen of the test and the control, D&I: heart of the test and the control, E&J: lungs of the test and the test and control group. Images were taken at 40x magnification.

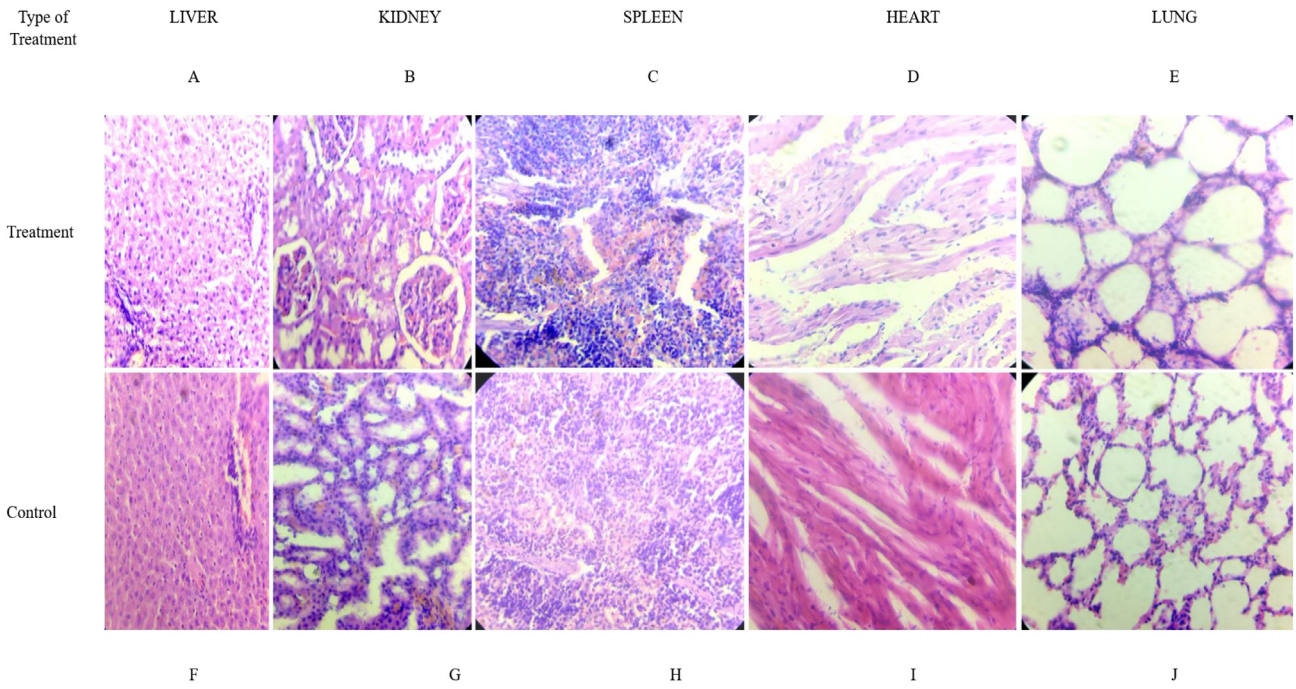

Figure 5. H&E histology Images for Wistar rats treated with Drs B2 (1.9, 4.6, and 9.3 mg/kg) after 90-day treatment. A&F: liver for the test and Control mice, B&G: Kidney for the test and the control, C&H: Spleen of the test and the control, D&I: heart of the test and the control, E&J: lungs of the test and the control group. Images were taken at 40x magnification.

treated peptide Drs B2. This finding shows an inflammatory response in the spleen which indicates or lead to systemic infection. In line with this result, Rodrigo *et. al* (2011) reported the presence of megakaryocytes in the spleen of mice treated with P34 peptide indicating an process in the spleen [11]. Another study by Puertollano *et al.* (2003) looked at the pro-inflammatory cytokines released in mouse spleen cells in response to nisin found that several cytokines implicated in inflammation were upregulated [32]. The same results were observed with the S-thanatin peptide, multinucleated giant cells increased and aggregated in the spleen of all groups, is attributed to a typical inflammatory response generated by intravenous infusion rather than a peptide side effect [13].

5. Conclusion

The results of this study indicated that intraperitoneal injection of Drs B2 had no obvious adverse effects on physiological and biochemical functions of BALB/c mice. Lower levels of serum ALT and AST in rats were found treatments compared to controls, and no morphological changes of this organ were observed. The presence of megakaryocytes shows an inflammatory response in the spleen of the mice and the rats. Therefore, these findings provide a reference for future toxicity studies and safety assessment of Drs B2 in non-human primates and clinical trials.

Declarations

Author contribution statement

Ahmed A. Abdille, Josephine Kimani, Esther N. Maina, Fred Wamunyokoli: Conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

Shedrack Reuben Kitimu: Analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

Mark M. Ndubi, Yahaya Gavamukulya: Analyzed and interpreted the data, analysis tools or data, wrote the paper.

Wallace Bulimo: Contributed reagents, materials, analysis tools or data; wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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