



UNIVERSITI PUTRA MALAYSIA

***EVALUATION OF ACUTE TOXICITY OF PURPLE SWEET POTATO
(IPOMOEA BATATAS) LEAVES ETHANOLIC EXTRACT ON KIDNEY
OF SPRAGUE DAWLEY RATS***

SITI SARAH HANUM BINTI SUHAIMI

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ABSTRACT

Evaluation of Acute Toxicity of Purple Sweet Potato (*Ipomoea batatas*) Leaves Ethanolic Extract on Kidney of Sprague Dawley Rats

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Introduction: Sweet potato (*Ipomoea batatas* L.) leaves are herbaceous plant widely eaten as a vegetable in African and Asian countries. Their extract has been proven scientifically for their efficacy as antioxidant, antimicrobial, and hypoglycemic agent. These promising beneficial effects have raised interest in sweet potato leaves' potential on human health but there is no univocal data on its safety or toxicity. It is commonly accepted that the kidneys are considered as one of the main target organs of toxicity. Hence, the assessment of kidney function is important in evaluating the plant extract toxicity. **Objective:** This study aims to evaluate the acute toxic effect of purple sweet potato leaves ethanolic extract (PSPLEE) on kidney of Sprague Dawley rats. **Methodology:** The acute toxicity assessment was carried out by dividing the male Sprague Dawley rats into one treatment (n=3) and one control group (n=3). A single dose of 2000 mg/kg bw of PSPLEE was administered orally to the experimental rats. Following the treatment, they were observed daily up to 14 days for any signs of adverse effects or mortality. At the end of 14 days, all animals were euthanized with ketamine-xylazine to collect blood samples by cardiac puncture for renal function test analysis. Then, they were dissected to collect tissue samples from the kidney for histopathological examination. The data were analyzed by using an independent t-test with p-value < 0.05 considered as statistically significant. **Results:** All rats treated with a single dose (2000 mg/kg bw) of PSPLEE revealed no toxicological signs or mortality in male Sprague Dawley rats. The renal function test results also showed no significant differences in the control and the treated groups. The organ relative weight of kidneys was also normal. On histopathological examination, there were no significant abnormal microscopic changes compared with the control. **Discussion:** Evaluations of plants toxicity can identify some complications that may be related to its use. In this study, a single administration of the PSPLEE of a dose of 2000 mg/kg bw did not induce any mortality and toxicity among treated Sprague Dawley rats. It was supported through absence of treatment-related adverse effects on all of the parameters observed. **Conclusion:** Findings from this study have shown that the oral administration of the purple sweet potato (*I. batatas*) leaves ethanolic extract does not exert any significant toxic effect in male Sprague Dawley rats. Thus, *I. batatas* leaves could be regarded as safe to be consumed and to be developed as therapeutic agents.

Keywords: Acute toxicity, *Ipomoea batatas*, ethanol extract, kidney, histopathology



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ABSTRAK

Pengujian Ketoksikan Akut Ekstrak Etanol Daun Ubi Keledek Ungu (*Ipomoea batatas*) Pada Ginjal Tikus Sprague Dawley

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Pengenalan: Daun ubi keledek (*Ipomoea batatas* L.) merupakan tumbuhan yang sering dimakan sebagai sajian sayur oleh masyarakat di negara-negara Afrika dan Asia. Ekstrak daun tersebut telah terbukti secara saintifik dapat berkesan sebagai agen antioksidan, antimikrob, dan hipoglisemia. Manfaat-manfaat ini telah meningkatkan tarikan terhadap potensi daun ubi keledek untuk kebaikan kesihatan manusia, namun masih tiada data yang jelas mengenai keselamatan atau ketoksikannya. Umumnya diterima bahawa buah pinggang dianggap sebagai salah satu organ sasaran utama berlakunya ketoksikan. Oleh itu, penilaian fungsi ginjal adalah penting dalam menguji ketoksikan ekstrak tumbuhan. **Objektif:** Tujuan kajian ini adalah untuk menguji kesan toksik akut ekstrak etanol daun ubi keledek ungu (*Ipomoea batatas* L.) (PSPLEE) pada ginjal tikus jantan Sprague Dawley. **Metodologi:** Kajian ketoksikan akut telah dijalankan dengan membahagikan tikus jantan Sprague Dawley kepada satu kumpulan yang diberi ekstrak (n=3) dan satu kumpulan kawalan (n=3). Satu dos 2000 mg/kg PSPLEE diberikan secara oral kepada tikus-tikus eksperimen. Seterusnya, pemerhatian terhadap semua tikus dilakukan pada setiap hari selama 14 hari untuk mengesan sebarang kesan buruk atau kematian. Pada hari terakhir, semua haiwan itu telah dimatikan dengan menggunakan ketamine-xylazine untuk mengambil darah melalui tebukam kardium bagi tujuan menganalisis ujian fungsi ginjal. Kemudian, tikus-tikus tersebut dibedah untuk pensampelan tisu ginjal untuk pemeriksaan histopatologi. Semua data dianalisis dengan menggunakan ujian-t tidak bersandar, di mana nilai-p < 0.05 dianggap sebagai signifikan. **Keputusan:** Semua tikus yang diberi satu dos (2000 mg/kg) PSPLEE tidak menunjukkan tanda-tanda toksik atau kematian. Keputusan ujian fungsi ginjal juga tidak menunjukkan perbezaan yang ketara di antara kumpulan kawalan dengan kumpulan yang diberi ekstrak. Berat relatif organ ginjal juga adalah normal. Dalam pemeriksaan histologi, terdapat tiada perubahan mikroskopik abnormal signifikan berbanding dengan kumpulan kawalan. **Perbincangan:** Penilaian ketoksikan tumbuhan dapat mengenal pasti beberapa komplikasi yang mungkin berkaitan dengan penggunaannya. Dalam kajian ini, pemberian PSPLEE dengan satu dos 2000 mg/kg tidak menyebabkan kematian dan ketoksikan pada tikus Sprague Dawley. Ia disokong melalui ketiadaan kesan buruk yang disebabkan oleh pemberian ekstrak pada semua parameter yang dinilai. **Kesimpulan:** Penemuan dari kajian ini menunjukkan

bahawa pemberian oral ekstrak etanol daun ubi keledek ungu (*I. batatas*) tidak memberikan kesan toksik yang signifikan pada tikus Sprague Dawley jantan. Oleh itu, daun *I. batatas* boleh dianggap sebagai selamat untuk dimakan dan dibangunkan sebagai agen terapeutik.

Kata kunci: Ketoksikan akut, *Ipomoea batatas*, ekstrak etanol, ginjal, histopatologi



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LIST OF ABBREVIATIONS

%	Percentage
µm	Micrometer
v	Volume
g	Gram
mg	Milligram
kg	Kilogram
bw	Body weight
PSPLEE	Purple sweet potato leaves ethanolic extract
LD ₅₀	Lethal Dose 50% (dose which kills 50% of the test population)
OECD	Organisation for Economic Cooperation and Development
IACUC	Institutional Animal Care and Use Committee
FDA	Food and Drug Administration
HCT	Haematocrit red blood cell volume
MCV	Mean corpuscular volume
MCHC	Mean corpuscular haemoglobin concentration
ALP	Alkaline phosphatase
AST	Aspartate transaminase
ALT	Alanine transaminase
SEM	Standard error of the mean
MAPK	Mitogen-activated protein kinase
NFκB	Nuclear Factor kappa-light-chain-enhancer of activated B cells

CHAPTER 1

INTRODUCTION

1.1 Background

People have looked for alternative remedies to eliminate injury and heal different illnesses since time immemorial. Up to 60,000 years ago, there are substantial precedents for using herbal medicines in treating disease and found to be corroborated in the use of medicinal plants for medication development (Summer, 2000). Plants have numerous active constituents such as secondary metabolites with multiple biological reactions, exhibiting their uses in different industries including pharmaceuticals (Sarrafchi et al., 2015; Rabiei & Rafeian-Kopaei, 2014; Dangl & Jones, 2001). Due to their effectiveness, safety, easy accessibility and minimal side effects in comparison to synthetic medications, participation in using medicinal herbs is growing globally. Over 80 per cent of the world's population currently relies on natural products derived from plants as a source of medicines to cure many human health issues (Arumugam et al., 2016; Akhtar et al., 2014; Kumara et al., 2012).

Nonetheless, recent studies have shown that several medicinal plants have manifested negative impacts (Nath & Yadav, 2015). This creates admonition regarding

the possible harmful effect that may be exerted from the use of such medicinal plants. For that reason, determining the potential toxicity of any therapeutic plant extract intended for clinical use, is a key component in evaluating its possible adverse effects.

Ipomoea batatas (Family Convolvulaceae), commonly known as sweet potato, is a perennial plant native to Central America (Zhao et al., 2005). According to tradition, its leaves have been utilized to treat many illnesses such as type 2 diabetes and oral inflammation (Pochapski et al., 2011; Abel & Busia, 2005). Besides, the stem part is used to cure prostatic disease (Emmanuel, 2010). Current pharmacological research have discovered that the extracts from purple sweet potato leaves possess favorable effects via antioxidant activity (Zhang et al., 2019), hypoglycemic action (Ogunrinola et al., 2015), suppressing cancer growth (Kurata et al., 2007), and antimicrobial activity (Chakraborty et al., 2018).

Despite several studies on the sweet potato leaves' pharmacological benefits have been well documented, there is no experimental evidence on its potential toxicity. It is also hard to find any reports on the safety of sweet potato leaves usage to treat various ailments. Therefore, this study was carried out to assess the acute toxicity of purple sweet potato leaves extract in Sprague Dawley rats.

1.2 Problem Statement

There is inadequate research on purple sweet potato leaves extract's potential toxicity. This creates an admonition regarding the possible harmful effect that may be exerted from its use. Therefore, the assessment for acute toxicity of PSPLEE is regarded as necessary.

1.3 Objectives

1.3.1 General Objective

To investigate the acute toxic effect of purple sweet potato leaves ethanolic extract (PSPLEE) on the kidney of Sprague Dawley rats.

1.3.2 Specific Objectives

The specific objectives were:

- a) To prepare the purple sweet potato leaves ethanolic extract (PSPLEE).
- b) To observe the behavioural signs of toxicity and physical changes in PSPLEE-treated Sprague Dawley rats throughout 14 days.
- c) To determine the renal function test of PSPLEE-treated Sprague Dawley rats.
- d) To evaluate the histopathological differences in the kidneys of PSPLEE-treated Sprague Dawley rats.

1.4 Hypothesis

It is hypothesized that the purple sweet potato leaves ethanolic extract (PSPLEE) at a dose of 2000 mg/kg bw will demonstrate no toxicity effects on the treated rats during the 14 days' observation period.

CHAPTER 2

LITERATURE REVIEW

2.1 Toxicity Study

Toxicology is defined as the study of the harmful effects of substances on living organisms (Costa and Teixeira, 2014). Paracelsus (1493-1541) postulated that “All substances are poisons; there is none which is not poison. The right dose differentiates a poison from a remedy” (Grandjean, 2016). This indicates the basic principle of toxicology whereby the dose expression is needed to determine toxicity. Observations from experiments that describe the relationship between dosage and adverse events are incorporated with information on human exposure to generate a safety management from harm. Moreover, an initial safe dose as well as dose range of an experimental drug could be identified through the toxicity assessment (U.S. Food and Drug Administration, 2013). In pharmaceuticals, the experiment outcomes of toxicity tests conducted on animals are usually used to integrate with the potential drug’s efficacy information. This is to determine whether the benefits would outweigh the risks of undesired harmful effects. The data may also imply the possible toxic impacts of substance exposure on human health that need to be monitored attentively (Rowan, 2015).

Prior to entering clinical trials, preclinical *in vivo* toxicity testing needs to be carried out to investigate the potential human risk. Since a drug may affect animals differently, two mammalian species of animal models, ideally rodents and non-rodents, are required by FDA for the preclinical studies (U.S. Food and Drug Administration, 2013). Organisation for Economic Cooperation and Development (OECD) has established standard guidelines and policies for toxicity assessment by focusing on 3R's principles: replace, reduce and refine, to make the animal experiments more humane while providing reliable data. This would help to confront some ethical issues in regard to animal use and deal with it by implementing the 3Rs.

2.2 Acute Toxicity Study

For any drugs intended for human use, acute toxicity studies in animals are generally compulsory. It determines the adverse effects that may result within 14 days after a single dose of test substance exposure (Parasuraman, 2011). Acute toxicity studies are also designed to identify the substance's mean lethal dose or LD₅₀, which is described as the dose lethal to half of the test animals. Ultimately, the acute toxicity data can be used to calculate the dosing regimens for further long-term investigations (Ochoa, 2013).

Acute systemic toxicity is calculated by administering a single dosage of the substance to rodents, often via inhalation, topical application, or orally. Pharmaceutically,

the major purpose of the research is to establish the nature and the extent of any acute toxic reaction, including the maximal non-lethal dose as well as acquiring pertinent data. The data obtained from these experiments would be useful to be extrapolated to humans for diagnostic and therapeutic fields, and also being used in determining doses for subsequent repeat-dose trials (Rispin et al., 2002).

2.3 Herbal Toxicity

In spite of traditional remedies becoming more desirable for therapeutic purposes, there are still concerns regarding its safety. Only around 10% of herbal medicines on the global market are properly regulated with recognized active ingredients. Diligent quality control processes are often not rigorously enforced (Winston, 2019). Most of these herbs are still unknown in terms of their active and harmful components. In order to disclose some risks that may come with the usage of these products, toxicity testing can be conducted to uncover such risks. As a result, the possible adverse consequences while using them as medication could be prevented. Moreover, a variety of plants emit toxic secondary metabolites as a defensive measure against danger. Some medicinal plants such as *Digitalis purpurea*, *Solanum nigrum*, *Atropa belladonna*, and *Podophyllum peltatum* are not distinguishable from their toxic elements (Kennedy & Wightman, 2011). Additional factor that contributes to particular plants' toxicity is the occurring poisonous minerals and heavy metals such as lead, mercury, cadmium, and arsenic (Dwivedi & Dey,

2002). When herbal products tainted with these metals are consumed, they might give rise to substantial neurological damage.

Hepatotoxicity is linked with majority of the toxicology investigations performed on medicinal plants (Bjornsson et al., 2013). Also, the adverse reactions on the circulatory system, kidneys, neurological system, and blood have been reported in medical publications, including the mutagenicity and carcinogenicity of the herbal medicines. Hence, various advanced biological experimental approaches were performed as baseline safety assessments before conducting the efficacy trials. The principal objective of any medicinal plant toxicity evaluation is to determine toxic effects and to establish the exposure thresholds at which these reactions can happen. The severity of the adverse impact, as well as the exposure level at which the impact is detected, are two crucial elements to consider when assessing the safety of any herbal medicine. Evaluations of plants toxicity can identify some complications that may be related to its use particularly in vulnerable groups. The identification of harmful herbal extracts or metabolites generated from the pre-clinical and clinical phases of drug discovery and development is an equally essential goal of toxicity assessment. This will make it easier to determine toxicants that can be eliminated or adjusted during the procedure, as well as allow for a thorough study of safer and better solutions (Gamaniel, 2000).

2.4 Sweet Potato (*Ipomoea batatas*)

Ipomoea batatas is commonly known as sweet potato or *ubi keledak* in Malaysia. This plant is an *Ipomoea* genus from the Convolvulaceae family. It is believed to be native in tropical regions of America and have widespread to other tropical, subtropical, and temperate areas, including East Asia, Oceania, and Sub-Saharan Africa (Nguyen et al., 2021). Sweet potato grows as a perennial crop whereby its tubers are mostly harvested for consumption and its flesh can be in a variety colours (purple, cream, yellow, orange, red, white). According to Bovell (2007), the distinctive flesh colours are due to their carotenoids content. Apart from the tuber, the leaves of sweet potato are also eaten as a leafy vegetable due to its high yield, drought tolerance, as well as being able to thrive in a variety of climates and agricultural methods.



Figure 2.1: Purple sweet potato leaves

Numerous research has been conducted to determine the effects of sweet potato leaves consumption in terms of medicinal potentials. Their phytochemicals have been explored to have multiple biological effects through *in vitro*, *in vivo*, and a few human studies. For instance, scientists from Taiwan revealed that the intake of polyphenol-rich purple sweet potato leaves by basketball players enhances their antioxidative activities by lowering the oxidative stress caused by exercise (Chang et al., 2007). In a series of experiments with non-insulin dependent diabetic rats, flavones isolated from the sweet potato leaves reduce the fasting plasma insulin, blood glucose level, and low-density lipoprotein cholesterol. Besides, the insulin sensitive index and superoxide dismutase

level were elevated in the rats. These results are believed to be an additional benefit from sweet potato leaves as an antidiabetic agent (Zhao et al., 2007).

Studies have also been conducted on the hepatoprotective ability of purple sweet potato leaves. In an evaluation performed by Wang et al. (2014), the administration of anthocyanins of purple sweet potato leaves on carbon tetrachloride-treated human normal hepatocyte HL7702 cells reduced the reactive oxygen species (ROS) level, proposing that there are potential of sweet potato leaves to be a hepatoprotective agent against liver cell damage. Studies have also shown that sweet potato leaves can be used to manage inflammation. It has been previously reported that cyanidin and quercetin found in sweet potato leaves cause reduction of NF κ B expression and MAPK signaling, thereby inhibiting inflammatory response (Chao et al., 2013).

Due to its haematinic effect, sweet potato leaves are also consumed in effort to treat anaemia (Osime et al., 2009). Recent evidence suggests that its haematological effects of increasing hematocrit, RBC and hemoglobin count are contributed by the high concentration of folic acid, iron, and vitamin B6 in the sweet potato leaves (Montejo et al., 2015). In countries like Malaysia, sweet potato leaves are easily obtainable and commonly served as vegetable dishes for its well-known potential health benefits.

2.5 Solvent for Extraction

Medicinal plants contain a large number of naturally occurring phytochemicals that have been shown to have several beneficial properties, such as antioxidant, anti-inflammatory, and anticancer activity. One of the most abundant classes of phytochemicals that play a significant role in plant physiology and morphology, may be found in polyphenols. Phytochemicals that make up this category have shown the ability to carry out biochemical processes. Moreover, the kind of solvent utilized is critical to the successful identification of biologically active molecules where it depends on the intended usage of the extract. For instance, a particular solvent is selected due to their ability to evaporate at low temperature, facilitate quick physiologic absorption, act as a preservation agent, and less toxic (Simon et al., 2015).

Since different solvents have various extracting capacities, choosing the right one for the extraction procedure is critical for toxicity assessment. The selected solvent may also affect the chemical or molecular interaction. Using the suitable solvent would enable thermodynamic and kinetic control over the chemical interaction as solvents may influence its solubility, stability, and the rate of reactions (Sasidharan et al., 2010).

In mild conditions, it is preferable to do extraction by using low-reactivity solvents (Ghisalberti, 2007). The most often employed solvents for extraction in this application

are ethyl acetate, ethanol, methanol, and acetone. On the other hand, when working with air or freeze-dried samples, it is best to use a polar solvent such as ethyl acetate or methanol.

Alcoholic solvents break cell membranes and draw out a higher proportion of intracellular substances, leaving the pure components for a subsequent chromatographic separation. If a combination of solvents is a better alternative for extracting particular compounds, a proportion of 1:1 of them is the great way to have a high extraction efficiency; however, the ratio depends heavily on the chosen solvents combination polarity (Wakeel et al., 2019). Studies have demonstrated that various solvents' extracts provide significantly varied LC_{50} values, chiefly because some solvents are less capable of yielding the particular bioactive compounds from the plant sample that contribute to toxicity (Lalisan et al., 2014).

2.6 Laboratory Test Animals

The study on animals which include regulating and exploring variables that influence their behaviour or biological system is referred to as in vivo testing. Oftentimes, animal testing is carried out at commercial institutions that offer industry with animal study services. Animal testing can be classified into two: primate and non-primate. Since primates are similar to humans physiologically, they are assessed for possible impacts on

organs in the nervous system, female reproductive system, and visual system. For toxicity study, it is frequently conducted on non-primate species, such as rodents, as they're more closely related to humans phylogenetically (Chellman et al., 2009).

Even though mice may be employed, it is generally recommended to utilize rats. It is biologically feasible for mice to react to toxicants the same way as rats do but dissecting their tiny organs will have much more technical difficulties, leading to a higher variability. Meanwhile, in terms of gender, male rats tend to have a greater sensitivity towards toxicological studies than female rats. Therefore, male rats are desirable for this kind of investigation (Lipnick et al., 1995).

The animals should be randomly allocated into control and treatment groups (Suresh, 2011). This aims to limit bias and ensures that significant variables are comparable between the groups. Ideally, 5-6 week-old rats with coequal weight are employed. The acceptable range of weight differences is within $\pm 20\%$ of their respective average weights. A minimum of five rats should be in each group. If any rats are sacrificed throughout the testing period, additional rats required for the assessments should be incorporated from the start. The nutritional needs of the animals should be fulfilled for proper growth and the diets can be supplied through *ad libitum* feeding. Following treatment, the animals are examined one by one immediately within the first 4 hours and then once daily for the next 14 days. Routine observations should be performed daily to monitor any signs of adverse effects or mortality. The timeframes at which these signs of

toxicity arise and soothe are critical, particularly when there is a propensity for the effects to be delayed. Every observation on each animal must be meticulously documented, inclusive of any changes in behaviour, eye colour, sleep pattern, skin, fur, as well as salivation.

2.7 Routes of Administration

The path by which the test substance is administered into the animals should be taken into consideration (Hirota & Shimizu, 2012). If the extract is given via an inappropriate route, the information on adverse events may be lacking. A critical component in deciding the route of administration is to identify whether the substance is being delivered locally, systemically, or parenterally. Depending on the experiment goals, the choice of a certain route may be affected by the legal regulations. Besides, the substance administration schedule will also vary based on the study objectives. For instance, in order to test toxicity, it is critical to understand at what time the system of the animal is metabolically active, so that the suitable timing to do the treatment is known (Smolensky & Peppas, 2007). Rather than blending the test substance in food or water, gavages are frequently utilized to administer specified volume or dosage to the animals. For safety testing, dosing orally is the most frequent method to administer an agent. The greatest volume that may be supplied through gavage in a single dosage is determined by the size of the test animal whereby for rats, normally the amount should not go beyond 1.0-2.0 ml/100g body weight.

2.8 Toxicology Evaluation

In toxicity testing, certain toxicological assessments must be taken into account. This includes analysis on weekly body weight, organs weight, haematological and biochemical results, as well as histopathological examination.

2.8.1 Weekly Body Weight

In order to acquire a better understanding on the effects of the test substance, data on food intake and body weight from each animal can be gathered to determine their cumulative body weight gain in relation to daily feed intake (Hoffman, 2002). This information would assist in studying the substance's impact on animal growth.

2.8.2 Organ Weight

Treatment with the test substance would frequently affect the organ weight, hence weighing the organ would give a good indicator of the related effects. The factors that influence in selecting the relevant organ for weighing in this toxicological research are through understanding the animal physiology and metabolism, as well as the substance's toxicokinetics and mechanism of action (Woolley & Woolley, 2008). Moreover, the data

of organ weight must be evaluated in conjunction with histopathology examination, gross findings, and clinical pathology. This is because the weight changes that are observed may not always be associated with the adverse effects.

2.8.3 Haematological and Biochemical Analysis

Haematological and biochemical analyses are performed to identify any function changes on the liver and kidney caused by the test substance. Prior to blood sampling, the animals should be fasted for 12-18 hours. For preclinical toxicology studies, the primary haematology tests are white blood cell count, differential white blood count, red blood cell count, platelet count, haemoglobin concentration, haematocrit red blood cell volume (HCT), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) (Arsad et al., 2013).

Lymphocytes and neutrophils are the most prevalent form of white blood cell in the rat's peripheral blood (Delwatta et al., 2018). Variations of white blood cell and differential white blood count may arise depending on the age of the animal. This variability of white blood cells may be associated with the process of immunocompetence and immunosurveillance. In male rats, an increased level of immunoglobulin may affect the secretory immune responses, in which this may be associated with a better

immunological competence in older animals as compared to in weanling rats (Ebersole et al., 1985).

Liver and kidney are vital organs in the body so analysis on them is critical in determining a substance's toxicity (Olorunnisola et al., 2012). The standard chemical analysis of serum indicated for liver includes alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT). At least two enzymes must be measured to assess hepatocellular health whereby AST and ALT are frequently monitored clinically as one of the diagnostic procedures (Alam et al., 2009). Although both of them are used to check for any damage on the parenchymal cells of liver, ALT is a more accurate biomarker of liver impairment than the AST. This is because an increased level of AST may, instead, be a sign of other organ damage (Shen et al., 2015).

Toxins derived from plants may have an adverse effect on the kidney organs and disrupt their physiological processes. These impacts can be discovered by comparing the values of urea and creatinine to the typically anticipated functioning of the renal. The nephrons may have been disrupted if the creatinine and urea values are shown to be high (Mukida & Eagles, 2010).

2.8.4 Histopathology Analysis

Histopathology examination is performed in conjunction with body weight and organ weight measurements. After the excess fat and adjoining tissue are being removed thoroughly, the dissected organs should be weighed promptly to avoid the consequences of organ weight drying. Prior to microscopic examination, the tissues usually undergo several steps consisting of tissue fixation, processing, embedding, sectioning as well as staining.

It is important to evaluate all tissues collected from animals in both the control and treatment groups. Lesion scoring technique can be used to assess the pathogenicity of the tissues either in gross or histopathological analysis (Timbermont et al., 2010). It can provide data for group comparison as well. The particular dosage should be investigated if any effects associated with the treatment are observed in the tissues.

The primary function of the renal is to filter and eliminate waste materials. However, these functions may be impaired as a result of exposure to a toxic test compound such as chemicals in plants (Debelo et al., 2016). Thus, kidneys are regarded as useful in toxicity assessments due to their sensitivity to harmful substances. Microscopical examination should be performed on the kidney's proximal and convoluted tubules to

observe any signs of cellular, granular, and protein casts. Besides, the Bowman's capsule as well as the glomerulus can also be observed to predict toxicity (Loha et al., 2019).



CHAPTER 3

METHODOLOGY

3.1 Plant Material

In this study, purple sweet potato leaves obtained from a commercial sweet potato farm located at Sungai Pelek, Sepang, Selangor, were used. The plant was identified by Institute of Bioscience, Universiti Putra Malaysia, where the specimen voucher was deposited (MFI 0188/20) for future reference. The leaves were air dried, pulverized, and extracted in ethanol/water (80, %v/v) by mixing 20g of purple sweet potato leaves dried powder with 200mL of undenatured ethanol/water (80, %v/v). The mixture was then agitated on an orbital shaker for 24 hours at 150 rpm at a temperature of 25°C, and further filtered through a Whatman filter paper No. 1. The residue was re-extracted again under the whole process for three times. All those filtrates were combined and evaporated to dryness by using a rotary evaporator set at 48°C, followed by oven-dried (45°C).

3.2 Experimental Animals

The study was carried out by using 6 healthy adult male Sprague Dawley rats weighing about 150-200g at the age of 7 - 8 weeks. The rats were obtained from an animal house of the Faculty of Medicine and Health Sciences, UPM. Prior to the experiments, the rats were acclimatized for a week and housed individually in polypropylene cages. Their housing conditions were maintained at temperature of 23-25°C with a 12-h light/dark cycle and humidity at around 58%. Standard rat pellets and water were provided *ad libitum*, however the rats were subjected to fasting overnight before the treatment. All procedures were in compliance with the ethical guidelines from Guide for the Care and Use of Laboratory Animals (2011). This study was approved by the Institutional Animal Care and Use Committee (IACUC) University Putra Malaysia (UPM/IACUC/AUP-R070/2020).

3.3 Acute Oral Toxicity Study

Acute oral toxicity was determined in rats using the guidelines of the Organization for Economic Cooperation and Development (OECD) 420 with minor modifications. The 6 male Sprague Dawley rats were divided into one treatment and one control group, with three rats in each group. PSPLEE was dissolved in distilled water and administered orally to each rat in the treated group with a single dose of 2000 mg/kg bw. Meanwhile, the

control group received water only as a vehicle. Following that, general behavioral changes of the rats were observed at every 1,2,4 and 6 hours, and daily thereafter, up to 14 days. Various changes in physical appearance, and visual observation were also conducted for any signs of acute toxicity. At the end of 14 days, all animals were anesthetized with ketamine-xylazine to collect blood samples via cardiac puncture into non-heparinized tubes for renal function test. Then, their tissue samples from the kidney were collected, weighed, and prepared for histopathological examination.

3.4 Renal Function Test

For renal function test, non-heparinized blood was first centrifuged at 3000 rpm for 10 min. Then the separated serum was used to analyze measurements of urea, creatinine, and ions (sodium, potassium and chloride).

3.5 Histopathological Analysis

The kidneys were carefully dissected out and weighed. They were then fixed in 10% formalin, dehydrated with ethanol, cleared in xylene and embedded in paraffin. A microtome was used to prepare 5 μm thick sections of sample, which then transferred to a warm water bath. The tissue ribbons were scooped up onto a slide and allowed to dry

upright at 37°C. They were stained with haematoxylin and eosin and observed under a light microscope for histopathological examination.

3.6 Statistical Analysis

All data of body weight, organ weight, and renal function test results were expressed as mean \pm SEM. The statistical differences among the means of groups were conducted by using independent t-test whereby results were considered as statistically significant at $p < 0.05$. A statistical software (SPSS statistics 20.0) was used to analyze the data.

CHAPTER 4

RESULTS

4.1 General Appearance and Behavioral Observations

No signs of toxicity and death were observed in all rats, particularly the rats in the treatment group (Table 4.1).

Table 4.1: General appearance and behavioral observations on rats in control and treated groups.

Observation	Control group	Treated group
Behavior	Normal	Normal
General physique	Normal	Normal
Skin and fur	Normal	Normal
Eye colour	Normal	Normal
Diarrhea	Not present	Not present
Sleep pattern	Normal	Normal
Salivation	Normal	Normal
Death	Alive	Alive

Treated group: 2000 mg/kg bw of purple sweet potato leaves ethanolic extract (PSPLEE).

4.2 Body Weight

The body weights of all rats are shown in Table 4.2. The body weight differences observed between the control and treated group throughout the study period were not statistically significant. There was also a gradual increase in body weight of both control and treated rats.

Table 4.2: Effect of oral administration of purple sweet potato leaves ethanolic extract (PSPLEE) on body weight (g) of Sprague Dawley rats.

Day / Week	Control group	PSPLEE 2000 mg/kg bw
Day 0	206.60 ± 8.13	210.20 ± 1.53
Week 1	250.40 ± 4.99	262.80 ± 2.03
Week 2	311.25 ± 9.22	301.80 ± 4.33

PSPLEE; purple sweet potato leaves ethanolic extract. Values are presented as mean ± SEM (n=3).

Weekly Mean Body Weight of Sprague Dawley Rats During Acute Toxicity Study of PSPLEE

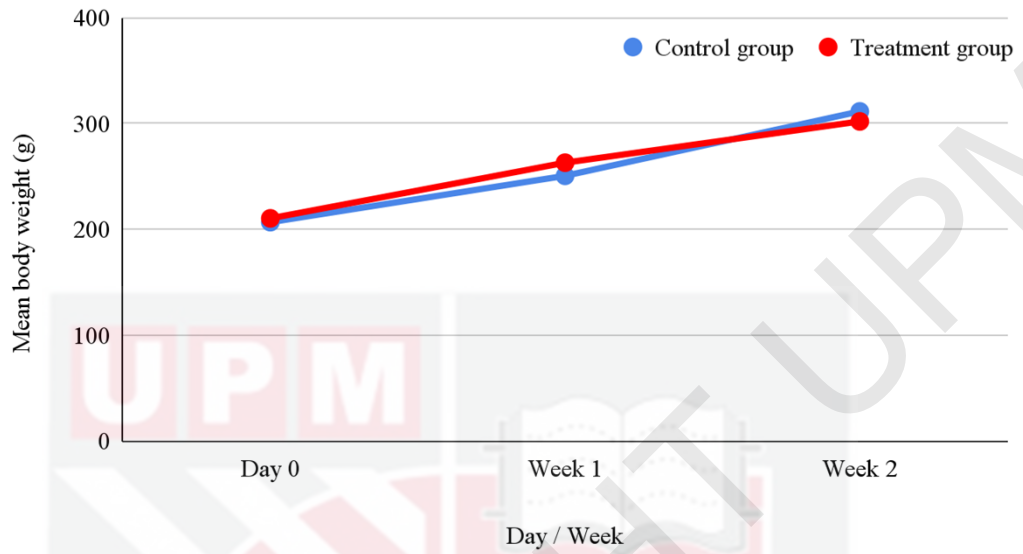


Figure 4.1: The effect of PSPLEE (2000 mg/kg bw) on mean body weight of Sprague Dawley rats.

4.3 Organ Relative Weight

There was no statistically significant difference ($p>0.05$) in organ relative weight of kidneys between control and PSPLEE-treated group at a dose of 2000 mg/kg bw. The effect of the PSPLEE on the organ relative weights of kidneys in the male Sprague Dawley rats are shown in Table 4.3.

Table 4.3: Effect of oral administration of purple sweet potato leaves ethanolic extract (PSPLEE) on relative organ weight of Sprague Dawley rats.

Parameters	Control group	PSPLEE 2000 mg/kg bw
Body weight (g)	292.67 ± 14.18	295.67 ± 9.43
Kidney weight (g)	2.12 ± 0.31	2.28 ± 0.18
Relative organ weight of kidney (%)	0.74 ± 0.13	0.77 ± 0.08

PSPLEE; purple sweet potato leaves ethanolic extract. Values are presented as mean ± SEM (n=3).

4.4 Renal Function Test

The results of the renal function test showed no statistically significant difference between the control and the treated rats. The readings fall within the reference range except on potassium values in both control and treatment groups. The effects of the tested plant extract on the renal parameters are shown in Table 4.4.

Table 4.4: Effect of oral administration of purple sweet potato leaves ethanolic extract (PSPLEE) on renal function test of Sprague Dawley rats.

Parameters	Reference range	Control group	PSPLEE 2000 mg/kg bw
Sodium (mmol/L)	132.1 - 170.4	145.00 ± 1.00	145.33 ± 0.88
Potassium (mmol/L)	2.8 - 5.8	10.00 ± 0.00	9.50 ± 0.50
Chloride (mmol/L)	87.7 - 106.6	97.00 ± 1.53	100.67 ± 1.45
Urea (mmol/L)	6.6 - 31.4	8.30 ± 0.21	7.60 ± 0.58
Creatinine (µmol/L)	26.5 - 70.7	51.00 ± 4.51	44.33 ± 4.41

PSPLEE; purple sweet potato leaves ethanolic extract. Values are presented as mean ± SEM (n=3).

4.5 Histopathology Findings

In histopathological evaluation, the kidney of rats treated with 2000 mg/kg bw of PSPLEE showed normal glomerulus and Bowman's capsule similar to the kidney of the control group that exhibited the normal histological features. The tubules also were found intact.

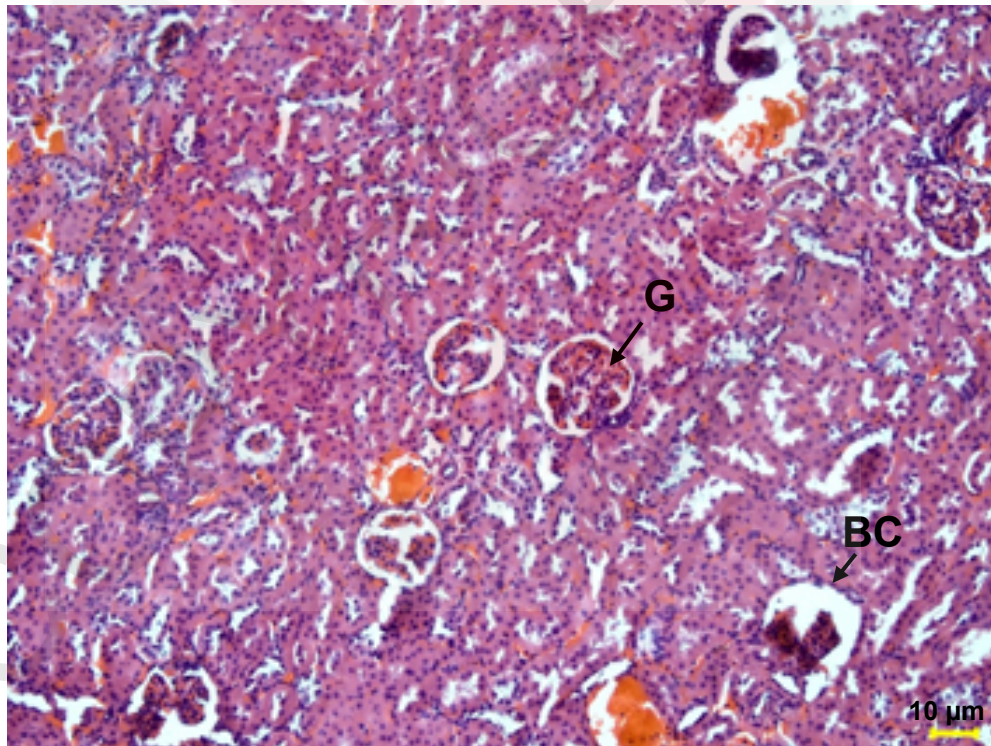


Figure 4.2: Representative photomicrograph of normal kidney section of a rat in control group (H&E stain, magnification: 100x). **G**; glomerulus, **BC**; Bowman's capsule.

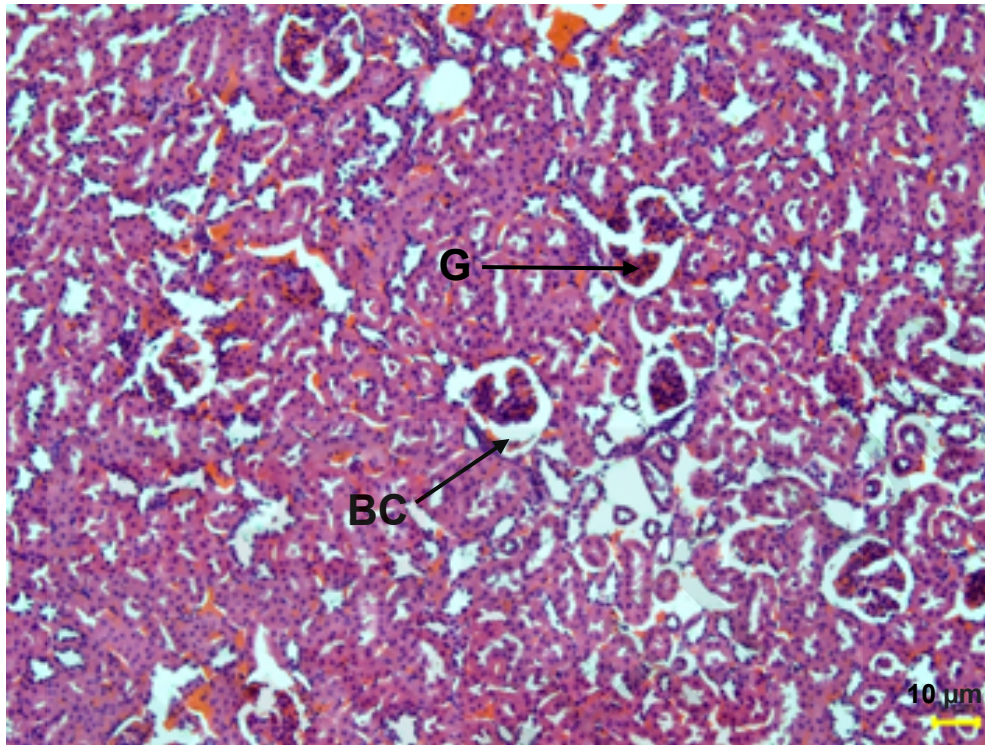


Figure 4.3: Representative photomicrograph of normal kidney section of a rat in treatment group (H&E stain, magnification: 100x). **G**; glomerulus, **BC**; Bowman's capsule.

CHAPTER 5

DISCUSSION

Plant origin drugs have become generally common and grown more popular across the world, including Malaysia. Due to its natural sources, they are believed to be harmless, without any adverse health consequences to humans (Hor et al., 2011). Moreover, researchers find medicinal plants to be an acceptable alternative to pharmaceutical medicines especially in treating a variety of chronic illnesses (Mythilypriya et al., 2007). However, toxicity can occur if the consumption of the herbal medicines is taken unsupervised, concerning their dosage. This is because there is still a dearth of scientific evidence to demonstrate the toxic effects of these remedies, causing the potential health risk unknown. Therefore, it is essential to evaluate the toxicity of the medicinal plants to ensure its safety before utilizing it as therapeutic use.

In the present study, purple sweet potato leaves ethanolic extract (PSPLEE) was investigated to determine its acute toxic effect on the kidney of Sprague Dawley rats. This plant has many scientific evidences on its pharmacological properties but is still lacking in terms of safety profile. The toxicity evaluation was conducted by administering a dose of 2000 mg/kg body weight orally to the rats and continuously monitored for 14 days. After the two weeks of treatment, the rats did not show major changes in behaviour and

abnormalities in general appearance. No mortality was also observed. Since clinical abnormalities are a significant indicator of organ damage in the treated-animals, PSPLEE can be considered as relatively safe on acute exposure as neither sign of behavioural changes nor abnormalities were produced. PSPLEE is also fairly harmless in single dose of 2000 mg/kg body weight considering the LD₅₀ seems to be higher than 2000 mg/kg. This is especially when any substance with an oral LD₅₀ more than 1000 mg/kg are regarded as non-toxic and less hazardous (Adeneye & Olagunju, 2009).

The change in body weight is an essential parameter for assessing a substance's toxicity (Vahalia et al., 2011). Throughout the study period, the mean body weight of both treatment group and control group increased gradually but the difference between them was not statistically significant. The increment may be due to body fat accumulation which may lead to an increase in animal's body weight (Arsad et al., 2013).

Organ relative weight is also a critical factor in evaluating the toxicity of a substance. It could help in determining whether the organ is damaged such as hypertrophy, swelling, as well as atrophy (Mohamed et al., 2011). Kidney is one of the vital organs for animal survival and directly involved in filtering out toxicants. Therefore, its relative ratio to body weight was evaluated in the present study. The kidney relative weight of the treated group compared to the control group was found to be not significant. This finding suggests that the PSPLEE did not affect the rats' kidney.

Serum biochemistry plays a key role as a marker in toxicological assessments due to their sensitivity to toxicant-induced clinical reactions. It can be used to analyze possible changes in organ function like kidney whether they are affected by the tested extract (Porwal et al., 2017). Serum urea and creatinine are commonly employed as a marker of renal function whereby they are usually elevated in renal disorder (Loha et al., 2019). Excretion of urea will slow down, resulting in an increase in plasma urea as the kidney is injured and unable to eliminate these chemicals at a regular pace. The same applies to creatinine where its concentration is regulated by glomerular filtration. Thus, creatinine clearance can be utilized as glomerular filtration rate indicators.

In this current study, a renal function test was performed and the results revealed that all parameters fall within the normal range except for potassium that exceeds the reference values (Petterino & Argentino-Storino, 2006) in both groups. This is most likely due to the feeding of high-potassium commercial chow. Linas and Sharma (2012) linked hyperkalemia to a high potassium intake, generally with poor renal potassium excretion. However, the observation seen were statistically insignificant, hence, PSPLEE was not evident enough to impair the renal in handling the electrolyte.

Furthermore, the administration of PSPLEE did not show significant differences in the urea and creatinine levels between the treatment and the control group. This indicates that the extract did not affect the morphological features of the kidney. According to Mirza and Panchal (2019), electrolyte balance is a measure of the kidney's

osmoregulation. Since the serum electrolytes (sodium, potassium and chloride) observed had no significant differences and was also corroborated with the absence of any abnormalities in the histopathological findings, it indicates that the PSPLEE did not disrupt the renal functioning.

Apart from that, the difference in the values of urea and creatinine between the treatment group and control group were apparent, where the readings were much lower in the treated rats. Although not significant, the reduction might be a result of phytochemical constituents. According to Vargas et al. (2018), flavonoids possess renoprotective effects and are able to enhance urea and creatinine clearance. Therefore, the most possible explanation for the reduction in the serum level of urea and creatinine in this study is due to the flavonoids content in the purple sweet potato leaves, which may have led to a higher clearance of these waste products in the treated rats.

Histopathological examinations were performed to establish the changes observed in the tissue. Moreover, cell injury may arise from the tested extract and their metabolites entering the organs (Debelo et al., 2016). Hence, histopathological examinations can assist in assessing toxic effects of the tested substance on tissues and organs that are associated with pathological alterations. In the current histopathological analysis of the kidney, PSPLEE-treated rats showed no significant changes in the microscopic structure of the kidney as compared to the control group. Their kidney architecture, appearance of the glomerulus, and Bowman's capsule are similar to the kidney of the control group that

exhibited the normal histological features. The tubules were also intact. Additionally, the results were supported by the renal function test that demonstrated statistically insignificant differences in the values compared to the control group. These findings imply that PSPLEE did not cause toxicological effects to the renal.



CHAPTER 6

CONCLUSION AND FUTURE RECOMMENDATION

6.1 Conclusion

In this present study, the purple sweet potato (*Ipomoea batatas*) leaves ethanolic extract did not exert any significant toxic effect in male Sprague Dawley rats with regard to the clinical observation, body weight and renal parameters. There was also no mortality observed throughout the 14 days after a single oral dosage of the extract was administered. Moreover, there were no toxicological signs in the treated rats' kidney sections. Thus, *I. batatas* leaves could be regarded as safe to be consumed and to be developed as therapeutic agents.

6.2 Future Recommendation

Some further experimental activities for a longer period such as chronic toxicity study is suggested to be carried out to confirm the safety profile before developing *I. batatas* leaves-based health product. Besides, increasing the dosage of the PSPLEE would be helpful for further research in assessing the toxic dose of PSPLEE. Moreover, other

organs such as brain, heart, liver, and spleen could be used to evaluate and set a clear picture regarding the margin of safety of the plant.



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A. Reference range value

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Table 3. Clinical chemistry control data from 4 and 13-week toxicity studies in male Sprague-Dawley rats

Parameters	Units	Males, 4 weeks			Males, 13 weeks				
		n	Mean \pm s.d.	Minimum value	Maximum value	n	Mean \pm s.d.	Minimum value	Maximum value
Albumin/globulin ratio		37	1.0 \pm 0.1*	0.5	1.1	10	0.7 \pm 0.1 [§]	0.6	0.8
Albumin	g/L	82	35.0 \pm 4.0*	24.0	44.0	90	34.0 \pm 3.0 [§]	29.0	41.0
Alanine amino-transferase	U/L	162	44.8 \pm 11.4*	19.1	78.0	160	53.7 \pm 18.0 ^{§*}	34.9	218.1
Alkaline phosphatase	U/L	162	340.6 \pm 113.5*	162.3	769.7	160	258.9 \pm 73.6 ^{§*}	131.6	459.0
Aspartate amino-transferase	U/L	162	92.6 \pm 31.3*	38.4	215.3	160	92.8 \pm 25.3 [§]	56.1	201.8
Total bilirubin	μ mol/L	162	1.71 \pm 0.1	0.0	5.1	160	1.7 \pm 0.3	0.0	5.1
Calcium	mmol/L	162	2.6 \pm 0.1	2.3	3.0	180	2.6 \pm 0.1	2.1	2.9
Total cholesterol	mmol/L	162	2.7 \pm 1.0	1.7	14.6	160	2.7 \pm 0.5	1.9	4.6
Chloride	mmol/L	162	96.3 \pm 3.9	87.7	106.6	160	97.0 \pm 4.9	81.5	104.0
Creatinine	μ mol/L	162	44.2 \pm 8.8*	26.5	70.7	160	53.0 \pm 8.8 ^{§*}	35.4	79.6
Gamma-glutamyl transferase	U/L	22	1.2 \pm 0.9*	0.0	3.2	—	—	—	—
Globulin	g/L	37	35.0 \pm 3.0*	29.0	47.0	10	43.0 \pm 3.0 [§]	40.0	49.0
Glucose	mmol/L	162	6.2 \pm 1.2	3.3	9.1	160	7.2 \pm 1.0 ^{§*}	5.0	11.2
Potassium	mmol/L	162	3.9 \pm 0.5*	2.8	5.8	180	3.8 \pm 0.5 [§]	2.9	5.3
Sodium	mmol/L	161	146.6 \pm 9.0	132.1	170.4	179	144.0 \pm 9.0 [§]	121.9	162.6
Inorganic phosphorus	mmol/L	22	2.5 \pm 0.2	2.0	2.8	—	—	—	—
Total protein	g/L	162	68.0 \pm 4.0	58.0	75.0	160	71.0 \pm 3.0 [§]	65.0	81.0
Triglycerides	μ mol/L	15	0.46 \pm 0.1	0.3	0.6	20	0.3 \pm 0.07 [§]	0.2	0.4
Urea	mmol/L	162	14.7 \pm 3.7*	6.6	31.4	160	16.2 \pm 4.4 ^{§*}	10.8	34.4

s.d., standard deviation.

* $p < 0.01$, vs. 4 weeks female group.§ $p < 0.01$, vs. 4 weeks male group.[‡] $p < 0.01$, vs. 13 weeks female group.

APPENDICES B

B. Raw data of renal function test in PSPLEE acute toxicity study

Group	Cage ID	Rat Id	Kill Id	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Urea (mmol/L)	Creatinine (μmol/L)
Acute Control	2	2	144	146	11	96	8.4	56
	3	3	145	146	11	100	8.6	42
	5	5	147	143	11	95	7.9	55
			Mean	145.00	11.00	97.00	8.30	51.00
			SD	1.41	0.00	2.16	0.29	6.38
			SEM	0.82	0.00	1.25	0.17	3.68

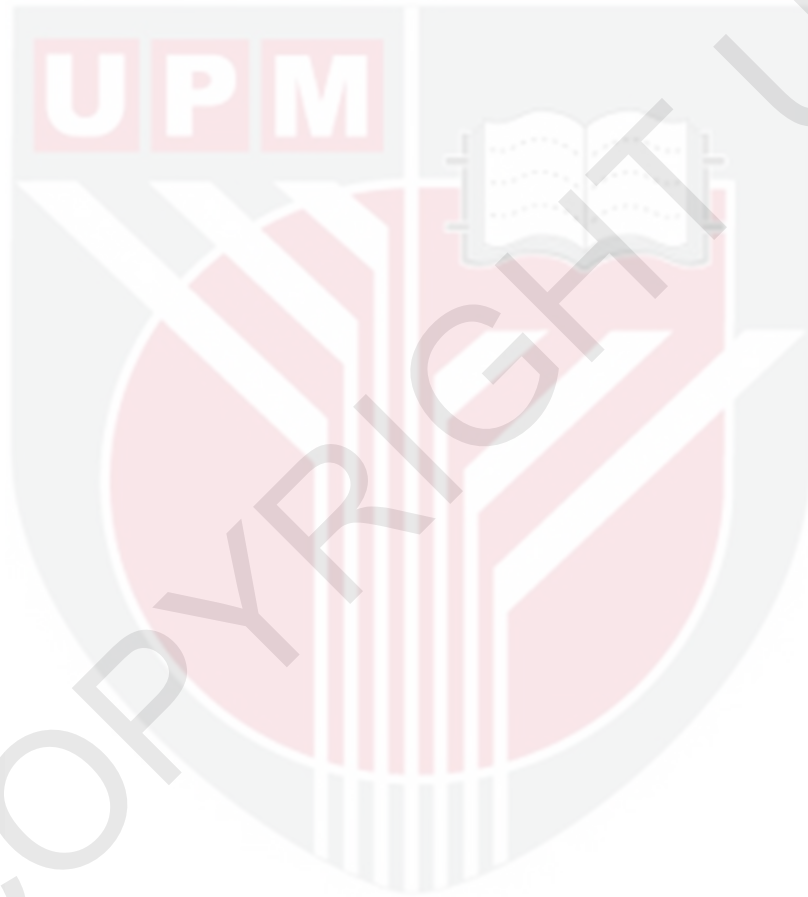
Group	Cage ID	Rat Id	Kill Id	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Urea (mmol/L)	Creatinine (μ mol/L)
Acute 2000mg/kg PSPLEE	1	1	148	144	11	103	7.6	51
	2	2	149	145	11	98	8.6	46
	5	5	152	147	8.5	101	6.6	36
			Mean	145.33	10.17	100.67	7.60	44.33
			SD	1.25	1.18	2.05	0.82	6.24
		SEM	0.72	0.68	1.19	0.47	3.60	

APPENDICES C

C. Raw data of body weight and relative kidney weight in PSPLEE acute toxicity study

Group	Cage ID	Rat Id	Kill Id	Body weight (g)	Kidney weight right & left (g)	Relative kidney weight right & left (%)
Acute Control	2	2	144	258	2.34	0.91
	3	3	145	308	2.52	0.82
	5	5	147	312	1.5	0.48
			Mean	292.67	2.12	0.74
			SD	24.57	0.44	0.18
			SEM	14.18	0.26	0.11

Group	Cage ID	Rat Id	Kill Id	Body weight (g)	Kidney weight right & left (g)	Relative kidney weight right & left (%)
Acute 2000mg/kg PSPLEE	1	1	148	276	2.32	0.84
	2	2	149	316	1.94	0.61
	5	5	152	295	2.57	0.87
			Mean	295.67	2.28	0.78
			SD	16.34	0.26	0.11
		SEM	9.43	0.15	0.07	



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