EFFECTS OF THREE-MONTH PARAQUAT EXPOSURES ON THE INTESTINE AND MAJOR ORGANS OF RATS: A HISTOPATHOLOGICAL ASSESSMENT

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by

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ABBREVIATION, ACRONYMS AND SYMBOLS

%	Percentage
&	and
Cm	Centimeter
DPX	Dibutylphthalate Polystyrene Xylene
EPA	Environmental Protection Agency
EU	European Union
H&E	Haematoxylin and Eosin
H20	Hydrogen peroxide
i.p.	Intraperitoneal
IHC	Immunohistochemistry
PD	Parkinson's disease
PQ	Paraquat
ROS	reactive oxygen species
SNpc	Substantia nigra pars compacta
US	United State
dH_2O	Distilled water

KESAN PENDEDAHAN PARAQUAT TIGA BULAN TERHADAP USUS DAN ORGAN-ORGAN UTAMA TIKUS: SATU PENILAIAN HISTOPATOLOGI

ABSTRAK

Paraquat (PQ) adalah racun herba yang digunakan di seluruh dunia kerana keberkesanannya yang tinggi dan aktiviti herbisida yang cepat bertindak. Walaupun demikian, penggunaan paraquat juga menyebabkan beberapa kesan negatif terhadap kesihatan manusia dan keselamatan alam sekitar. Banyak penyakit kronik seperti penyakit Parkinson, dermatitis, gangguan fungsi paru-paru, dan kanser telah dikaitkan dengan pendedahan paraquat dalam kajian epidemiologi. Walaupun begitu, kesan negatif ini belum dapat dibuktikan kerana kekurangan model haiwan yang sesuai dalam melakukan kajian kronik yang dapat mensimulasikan penyerapan PQ yang perlahan dalam keadaan harian. Oleh itu, kajian awal ini bertujuan untuk menganalisis kemungkinan penyerapan PQ pada organ utama selepas pengambilan makanan yang telah dirawat dengan PQ selama 3 bulan. Empat kumpulan tikus diberi diet normal dan makanan yang dirawat PQ pada dos yang berbeza iaitu 30 mg/Kg, 60 mg/Kg dan 100 mg/Kg, masing-masing. Setelah menjalani rawatan makanan selama tiga bulan, organ-organ utama tikus (peparu, hati, buah pinggang, dan usus) yang berkaitan dengan toksisiti paraquat dikumpul dan dianalisis memalui histopatologi konvensional dan perwarnaan imuno. Dari analisis histologi H&E, perbezaan yang tidak ketara antara organ-organ utama kumpulan kawalan dan yang menerima makanan yang mengandungi PQ. Walaupun begitu, dari analisis pewarnaan imunohistokimia, mungkin terdapat peningkatan yang signifikan dalam pemendapan paraquat pada organ-organ utama, terutama tisu usus di mana ia merupakan lapisan terdepan terhadap

makanan yang mengandungi PQ. Peningkatan dos PQ mungkin akan juga meningkat isyarat pewarnaan imunohistokimia. Kesimpulannya, PQ dapat diserap dan dikesan dalam tisu hidup setelah pengambilan makanan yang telah dirawat PQ. Kesan PQ pada tisu sekitarnya dapat disiasat pada masa depan.

EFFECTS OF THREE-MONTH PARAQUAT EXPOSURES ON THE INTESTINE AND MAJOR ORGANS OF RATS: A HISTOPATHOLOGICAL ASSESSMENT

ABSTRACT

Paraquat is an herbicide that was use worldwide due to the high cost-effectiveness and fast acting herbicidal activities. Nonetheless, the use of paraquat has also led to several health and environmental adverse consequences. Many chronic diseases such as Parkinson's disease, dermatitis, impaired lung function, and cancer have been associated with paraquat exposure in epidemiological studies. Nonetheless, the causal effect is yet to be proven due to the lack of a appropriate animal model in performing the chronic studies which could simulate the slow PQ absorption under normal daily condition. Hence, this preliminary study aimed at analyzing the possible absorption of paraquat (PQ) in major organs flowing a 3-month PQ-treated diet. Four groups of rats were treated with normal diet and PQ-treated diet at different doses i.e. 30 mg/Kg, 60 mg/Kg and 100 mg/Kg, respectively. Upon the three months diet treatment, the major organs of the rats related to paraquat toxicity were harvested and subjected to conventional and immune-histopathological analysis. From the H&E histological analysis, non-significant differences among the among the major organs between the control and PQ-diet groups were anticipated. Nonetheless, from the immunohistochemical staining analysis, there might be a significant increase in the paraquat deposition or infiltration in the major organs, particularly the intestinal tissues wherein it is the frontline layers exposure to the PQ-diet. There might be a dose-dependen relationship between PQ doses and the immunohistochemical staining signal.

In conclusion, the PQ-treat diet could be absorbed and detected in the living tissues following a PQ-treated diet. Effect of the toxic substances to the surrounding tissues could be investigated in future study.

CHAPTER 1

INTRODUCTION

1.1 Paraquat

Paraquat (1,1'-Dimethyl-4,4'-bipyridinium dichloride) is a nonselective contact herbicide commonly used to control broad leaf and grassy weed. It is one of the most used herbicides globally. Paraquat was first synthesized in 1882 as a redox indicator. Its herbicidal property was only recognized later in the 1950's and was introduced to the market as herbicides in early 1960's (Sittipunt, 2005). Paraquat is produced in several countries, including China, Province of Taiwan, Italy, the United Kingdom, and the USA and are used in more than 130 countries world-wide. Paraquat is labor saving and cheap, and therefore especially popular and accessible to farmers in developing countries (Wesseling *et al.*, 2001). The chemical structure is as shown in Figure 1.1.

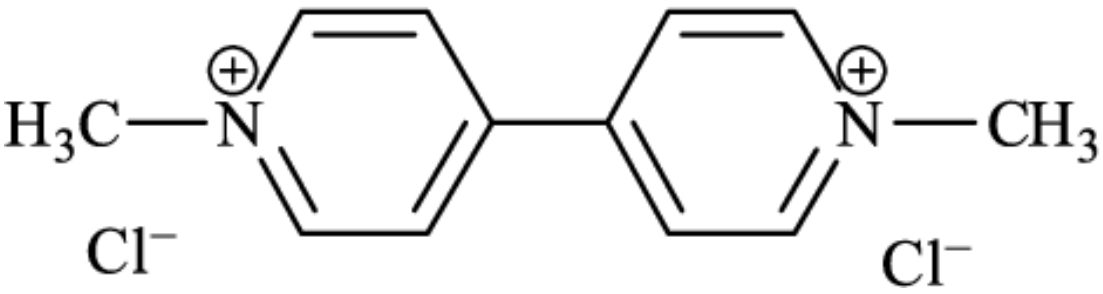


Figure 1.1 Molecular structure of PQ

They are also used at home and at work to ensure a pest-free environment. As a result of these, many problems such as unsafe use, persistence in environment, toxicity to bees, fish and wild life, contamination of water sources, persistent pesticide accumulating in food chain, negative impact on earthworms and other beneficial organisms has been identified. In developing countries, pesticides are routinely used

in unsafe conditions and farmers lack training and resources to increase safety to their own health and environment (Akinloye *et al.*, 2011).

The minimum lethal dose of paraquat is stated to be about 35 mg/kg body weight for human (Tang *et al.*, 2017b). The Symptoms of poisoning depend on the dose absorbed. It is difficult to estimate the dose absorbed from case histories since in many cases the patients spat out part of the paraquat concentrate or vomited profusely after swallowing the herbicide. Some patients have survived after apparently ingesting 50 - 100 mL Gramoxone(R) (10 - 20 g paraquat), whereas some died after taking as little as 2 sachets of Weedol (2.5 g paraquat) (WHO, 1984). Furthermore, absorption of an ingested dose of paraquat may be reduced by gastric lavage, induced emesis, whole-gut lavage or by the oral administration of adsorbent substances (Suntres, 2018).

Figure 1.2 Paraquat and its function in agriculture

According to(Choi, 2013 #149), Globally, there are about 20,000 annual fatalities and more than 2 million hospitalizations due to poisoning by pesticides and

agricultural chemical (Sittipunt, 2005). The fatality rate of PQ intoxication remains high due to the lack of an effective treatment. Current treatments include adsorbents, pharmacological approaches, radiotherapy, extracorporeal therapy, and immunosuppressive therapy, but the effectiveness of these therapies remains controversial. It is generally known that, the very high case fatality of paraquat is due both to its inherent toxicity and the lack of any effective treatment (Gawarammana and Buckley, 2011).

The restricted paraquat could add to the social distress associated with high suicide rates among subsistance farmers, by banning an essential tool to feed their families and enhance their prosperities. Paraquat has been banned or restricted in a number of countries. The US Environmental Protection Agency (EPA) allows its purchase and use solely by certified applicators. Paraquat is prohibited in Sweden, Finland, and Austria based on acute toxicity and absence of antidote. In Norway, the manufacturers canceled voluntarily its registration. In Germany and in The Netherlands, paraquat was banned because of its persistence in soil. The ban was subsequently lifted. Paraquat is being reviewed in the European Union and is in use in 10 of the 15 EU member states (http://europa.eu.int, status of current authorizations in December 2000) (Wesseling *et al.*, 2001). In Malaysia, the use of paraquat was banned in 2005 but was reintroduced into the market a year later and is currently available as a result of frequent and serious poisoning involving this agent. The number of calls relating to paraquat exposure when it was banned was 67 (36 and 31 in 2005 and 2006 respectively). After its re-introduction, there was a marked increase in the number of cases: 39, 79 and 101 for 2007, 2008 and 2009, respectively (Sazaroni *et al.*, 2012).

1.2 Paraquat Toxicity

Despite agriculture benefits, paraquat is extremely toxic to both humans and animals. Death cases due to intentional and accidental poisonings are frequently reported worldwide. Most incidents are caused by ingestion of the concentrated solution intended for agricultural use. Local effects include damage to the skin, nails, mouth, eyes and nose. Sore throat, dysphagia and epigastric pain may occur. Systemic effects, which produce the fatal outcome seen in those who have ingested a sufficient quantity of paraquat, mainly involve the respiratory system. The changes in the lungs that underly the symptoms and clinical signs comprise a proliferative alveolitis similar to that seen in most experimental animals treated with paraquat. In most, but not all, patients who develop the characteristic lung changes, the condition progresses inevitably towards a fatal outcome, death being due to respiratory failure. If overwhelming dose is taken, death is usually due to multi-organ failures. Numerous therapies have been tested, but none has been consistently successful (Marrs, 2003). The high mortality rate observed following paraquat exposure has been attributed to the lack of an antidote or effective treatment to ameliorate the toxic effect of poison (Gawarammana and Buckley, 2011). PQ induces its toxic effect mainly via oxidative stress–induced mechanisms (Suntres, 2002; Suntres, 2018).

1.3 Toxicokinetics of paraquat

Meredith and Vale (1987) demonstrated that, in human, PQ is absorbed incompletely from the gut and, it has been estimated that less than 5% of an ingested dose is absorbed over a 1-6-h period. Upon absorption, PQ is rapidly distributed via bloodstream to the whole-body systems. Under normal circumstances, the storage is not prolonged in any of the organ tissues, as it is being excreted by the kidney. Absorbed PQ mainly

eliminated as parent compound within 24 hours if the function of kidney is normal. In case of high dose ingestion, disturbance of normal kidney function is disturbed and may cause acute tubular necrosis which eventually affect the elimination process as well as prolonged the deposition of PQ in the tissues. Consequently, it can lead to multiorgan injuries and death which usually occur within hours and days. At moderate doses, delayed death due to lung fibrosis and respiratory failure usually occurs within weeks and month. Due to the structural similarity with naturally occurring polyamine, PQ is selectively absorbed and accumulated in the lungs, and exerts it major toxic effect in the lungs.

1.4 Mechanism of Toxicity

The cellular toxicity of paraquat is primary due to its redox cycle (Figure 1.4). It includes a well-known cascade of molecular reactions leading to NADPH consumption and to generation of reactive oxygen species (ROS) i.e. primarily hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). These ROS causes deleterious effect on affected tissues (Akinloye *et al.*, 2011). PQ is metabolized by several enzyme systems (NADPH-cytochrome P450 reductase, xanthine oxidase, NADH-ubiquinone oxidoreductase and nitric oxide synthase). Its metabolism through these systems generates a paraquat mono-cation radical (PQ^+). Inside the cell, PQ^+ rapidly gets re-oxidized to PQ^{2+} and in the process it generates superoxide (O_2^-). O_2 acts as an electron acceptor and NADP as an electron donor in this reaction. Generation of highly reactive oxygen and nitrite species results in toxicity in most organs but the toxicity is particularly severe in the lungs as paraquat is taken up against a concentration gradient in to the lung (Gawarammana and Buckley, 2011).

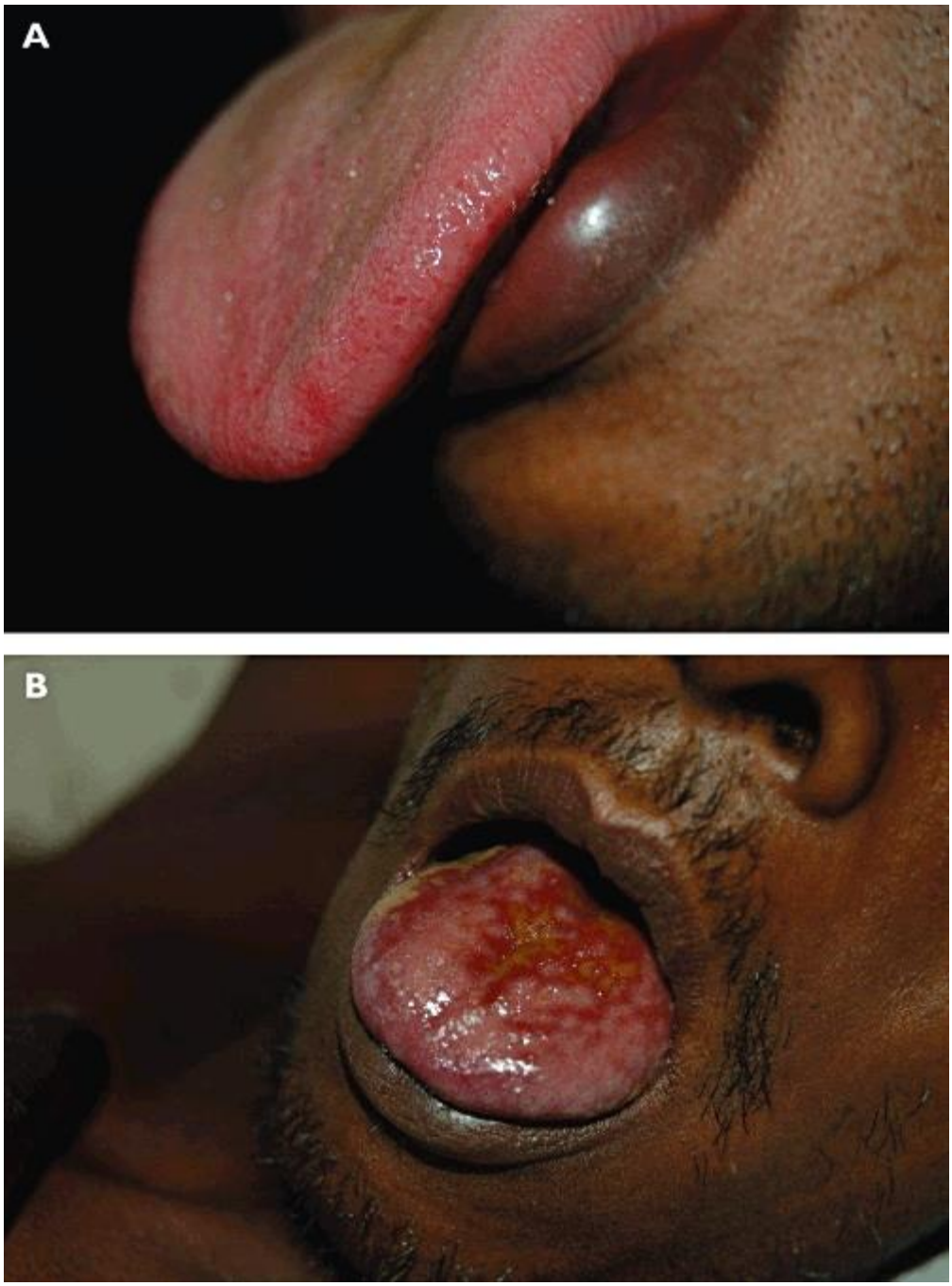


Figure 1.3 Consequences of paraaquat upon ingestion

Note:

(A) Paraquat tongue' early lesion, within 24 h after ingestion. (B)Paraquat tongue late lesion, 2 weeks after ingestion with extensive ulceration (Gawarammana and Buckley, 2011)

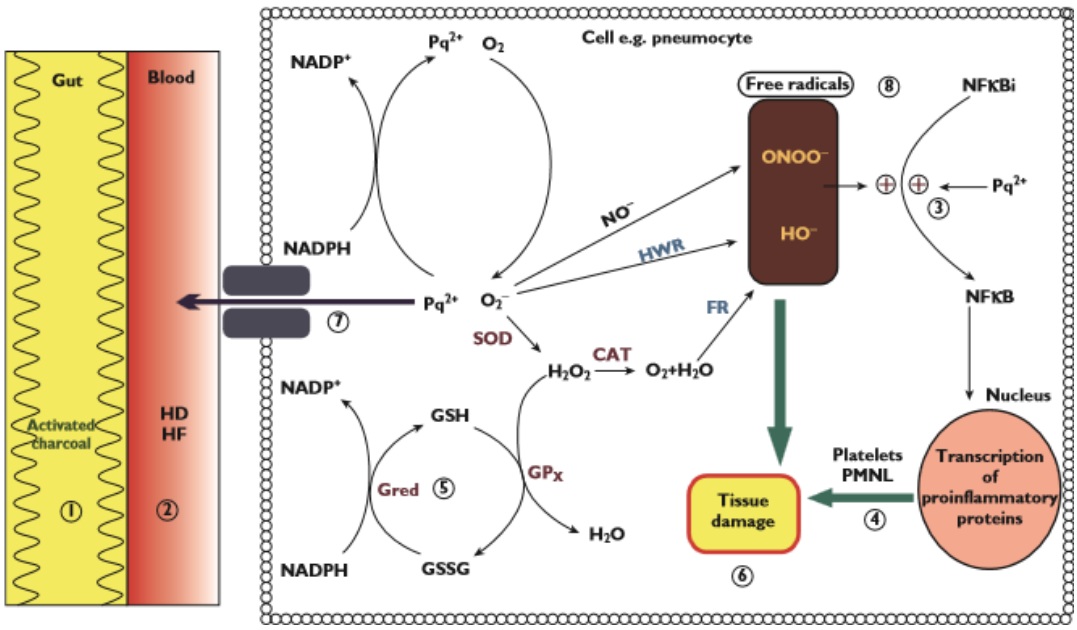


Figure 1.4 Graphical representation of paraquat toxicity inside a pneumocyte and potential sites of antidotal therapy.

Note:

SOD, superoxide dismutase; CAT, catalase; Gred, glutathione reductase; Gpx, glutathione peroxidase; FR, Fenton reaction; HWR, Haber-Weiss reaction.1–8: potential sites of action by available treatment options.1: activated charcoal and Fuller's earth;2: dialysis;3,4,6 and 8: salicylates;5 and 8: N-acetylcysteine;7 (P-glycoprotein induction): dexamethasone; 4: immunosuppression (Gawarammana and Buckley, 2011).

1.5 Analysis Assay

Paraquat detected from human blood, urine, and feces, separated on a strong acid cation exchange resin, reacted with Sodium dithionite, and determined spectrophotometrically at 391 nm. Paraquat ion can be determined with Na diothionite in clear urine, 1 pg/mL , and cloudy urine, 1.5 pg/mL . Urine can be passed through a Dowex AG 50W-X8 resin column either untreated or treated with 18N HzS04 or 25% trichloroacetic acid. The column was eluted with 2.5% NH4 C1 and the eluate reacted with Na dithionite to produce the blue free radical, which was determined spectrophotometrically at 392, 396, 400, and 401 nm. The method was sensitive to 0.01 pg ion per mL in a 250 mL aliquot of urine. Gas-liquid chromatography (GLC) cannot be used with paraquat because the polar nature of the chemical makes its extraction and concentration by organic solvents impossible, and results obtained by this method must be viewed with suspicion (Haley, 1979).

Generally, chromatographic and spectrophotometric methods for determination of paraquat in human tissues showed that the earlier method could not be used because of the presence of large amounts of contaminations. Even though, Paraquat is not concentrated by liver, kidney, or brain but achieves a lung concentration 10 times that of rat plasma, so the main target is lung more than other organs. In vitro, paraquat has no effect on lung surfactant, but in vivo surfactant is severely reduced or absent (Gawarammana and Buckley, 2011).

1.6 Chronic Toxicity

Chronic health effects of paraquat toxicity is associated with increased risk of Parkinsonism, it may relate to genetics, environmental, or both (Peng et al.,

2009)Generally, exposed patients suffer from loss of neurotransmitter cells (dopamine cells). Moreover, diffusion tensor imaging (DTI) revealed deficiencies in fractional anisotropy (FA) of substantia nigra (SN) when compared to normal people, which are similar parameters with non-exposed PD patients. Not to mention that the ROS activity of PQ can inflect oxidative stress on the cytosol and mitochondrial matrix of neuroblastoma cells (Rodriguez-Rocha et al., 2013). Also, low dosage poisoning over a long period of and iron exposure can increase the risk of PD. Chronic respiratory defects, it can cause gas exchange abnormalities in the lung (Schenker et al., 2004). de Jong et al. (2014) reported that PQ can gradually reduce lung function in proportionate with time. Additionally, PQ can impair the immune system by reducing the proliferative responses of T and B lymphocytes, reducing IgM plaque-forming cell counts, Inhibition of antigenic responses of B cell, and TH17 cells related autoimmune diseases (Hassuneh et al., 2012) Also skin exposure to PQ can cause dermatitis and severe skin irritation. Reproductive and Endocrine toxicity due to PQ is also reported by studies. In Malaysia, a significant reduction in semen quality among farmers who use PQ (Hossain et al., 2010). Among workers who had been in contact with PQ, a reported increase in birth defects in children. On animals, PQ can affect embryonal development (Hausburg et al., 2005). Genetic alterations were also been reported due to PQ. Mothers being exposed to PQ can cause leukemia in their children (Monge et al., 2007). Other malignancies also have been reported due to PQ, brain cancer (Engel et al., 2005), non-Hodgkin's lymphoma (Park et al., 2009), skin melanoma (Dennis et al., 2010), and breast cancer (Engel et al., 2005).

1.7 Toxicological Studies on Animal Models

Animal models were commonly used for toxicological studies of potentially harmful substances to support the potential anticipation of similar effect on humans. The

toxicological effect of paraquat on animals such as rat is evidence by the survival and histopathological findings on major organs such as lungs, liver, kidney and intestine (Choi *et al.*, 2013; Zhi *et al.*, 2011).

1.7.1 Lung Pathohistological Studies

According to Choi *et al.* (2013), the major abnormal or pathological findings of PQ toxicity related to lungs were the of increased alveolar wall thickness, hemorrhage, or cellular infiltration, while the control were showing thin alveoli epithelial with porous air sacs (Figure 1.5). Similar studies by Zhi *et al.* (2011) revealed worsening lung interstitial edema and widespread inflammatory cell infiltration in the alveolar space and septum, as well as fibrosis in PQ treated rat, from day-7 tissue to day-28 tissue (Figure 1.6).

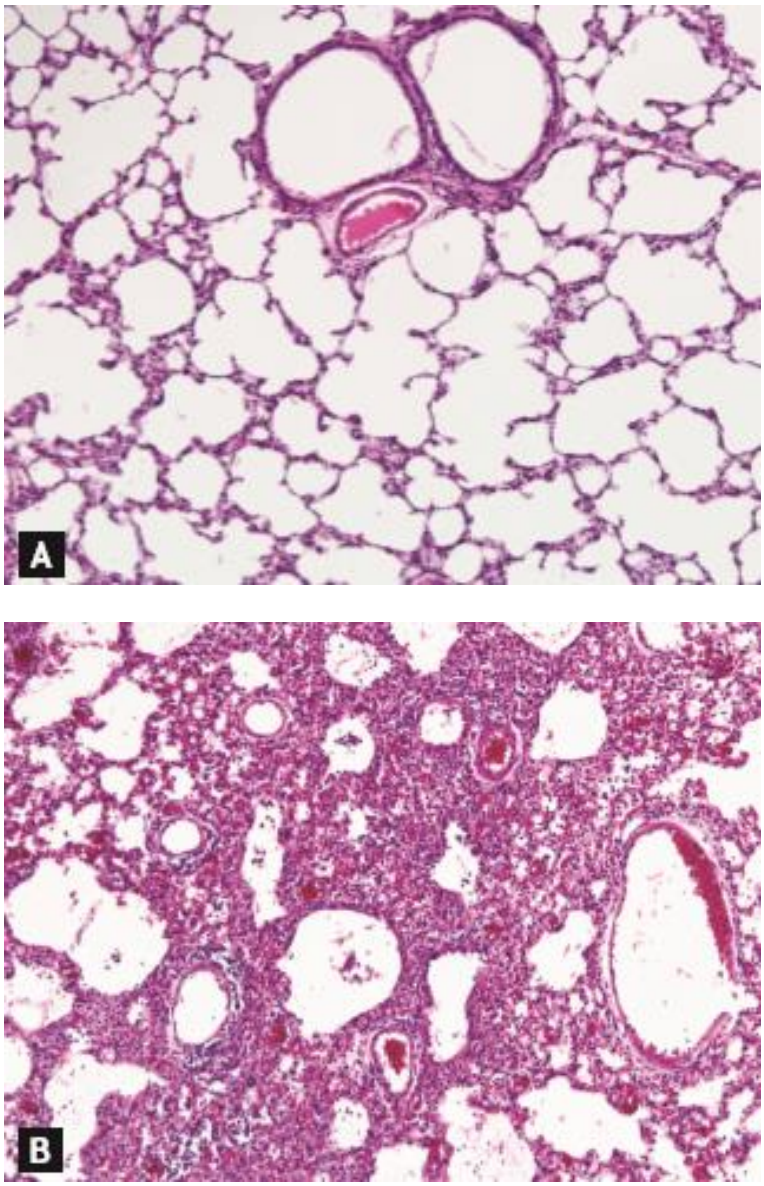


Figure 1.5 Histological comparison of lungs with and without paraquat treatment

Note:

Photomicrographs of lung sections stained with H&E (original magnification × 100) (Choi *et al.*, 2013). (A) Healthy control with a normal lung structure and no evidence of increased alveolar wall thickness, hemorrhage, or cellular infiltration; (B) Paraquat injection (35 mg/kg) only, with numerous inflammatory cells infiltrating the alveolar septum and spaces together with hemorrhage and congestion.

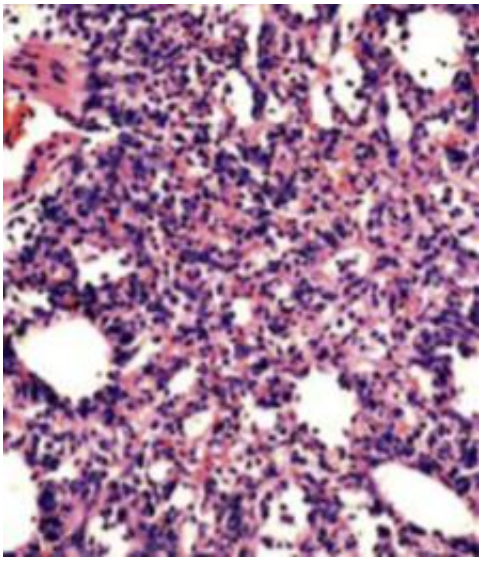
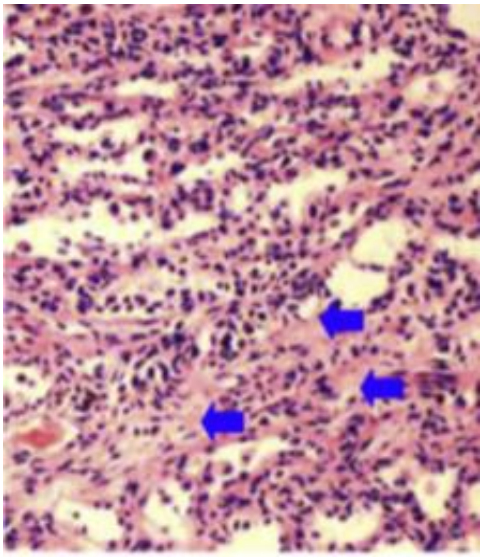
A.  B. 

Figure 1.6 Lung pathalogical findings in rat treated with PQ

Note:

Histological evaluation of rat lungs with paraquat hematoxylin and eosin staining (H&E×200). PQ intoxicated rat lung on day-7 (A) showed acute injury with interstitial edema and widespread inflammatory cell infiltration in the alveolar space and septum, whereas, PQ intoxicated rat lung on day-28 (B) showed widespread inflammation in alveolar spaces and septum and evident fibrosis was seen (blue arrows represented deposited collagen).

1.7.2 Liver Pathohistological Studies

Histopathological examination of liver section from PQ group rats revealed congestion in central vein. Granular and vacuolar changes near sub-capsular area with thickening of glisson's capsule noticed (Figure 1.7 and Figure 1.8). Section also revealed prominent reticular cells in the hepatic lobules and proliferation of bile ducts, suggesting hyperplasia of bile ducts. Sections from PQ group rats revealed granular and prominent vacuolar changes in the parenchyma of liver and congestion in central veins Focal areas of necrosis around central vein and periportal hyperplasia of bile ducts were noticed. In few sections, central lobular area revealed atrophy of hepatocytes with necrosis.

1.7.3 Kidney Pathohistological Studies

Sections of kidney from rats1 revealed congestion, focal hemorrhages, desquamation of tubular epithelium in lumen, decrease in glomerular size and tubular nephrosis (Figure 1.9). Sections from PQ group rats showed severe congestion, decreased in size of the glomeruli, hemorrhages, granular and degenerative changes in the tubular epithelium and desquamation in the lumen. Dilated tubules, cloudy swelling, vacuolar changes and necrosis of tubular epithelium results in occlusion of the lumen were noticed (Figure 1.10 and Figure 1.11)

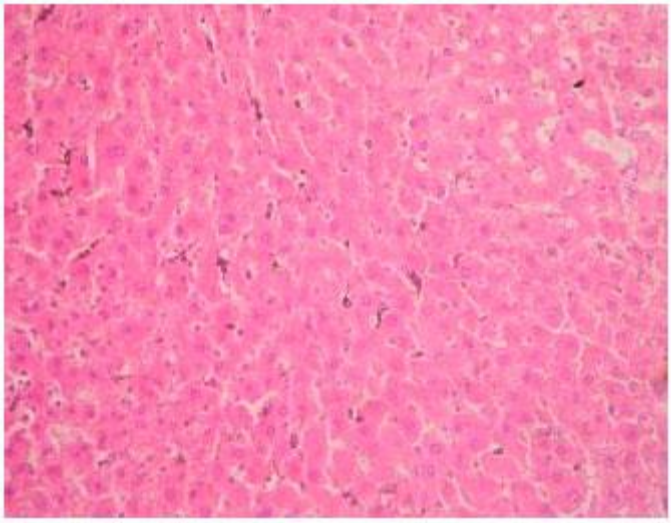


Figure 1.7 Section of liver from group-II rat showing granular and vacuolar changes and reticular cell hyperplasia. H and E stain 200X

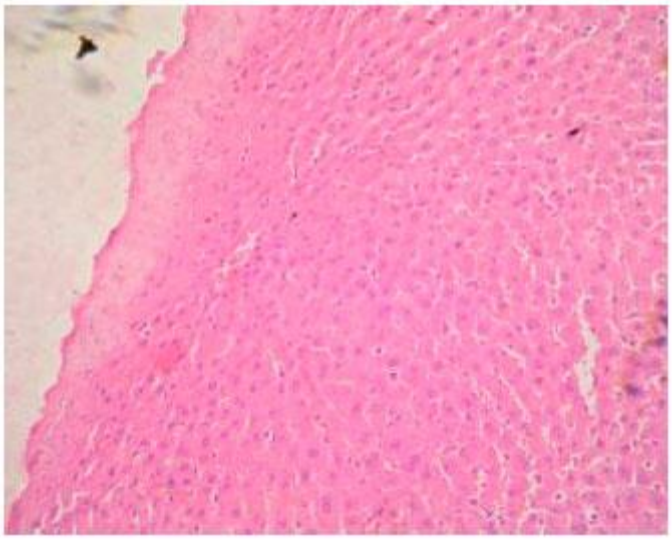


Figure 1.8 Section of liver from group-II rat showing thickened Glisson's capsule. H and E stain 200X

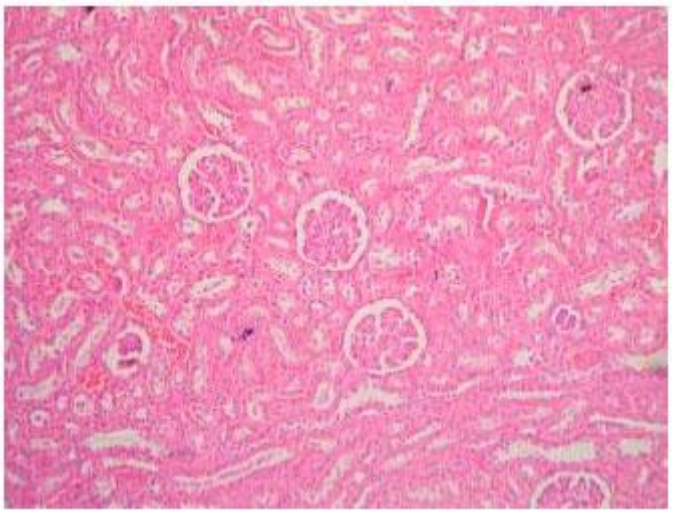


Figure 1.9 Section of kidney from group-II rat showing severe venous congestion. H and E stain 100X

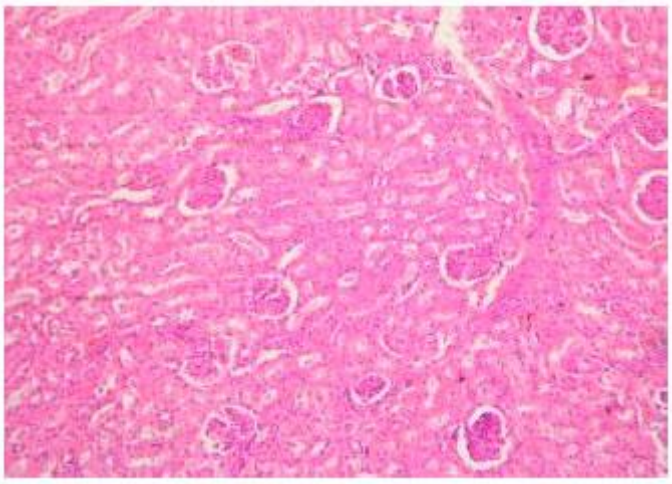


Figure 1.10 Section of kidney from group-III rat showing tubular nephrosis and venous congestion. H and E stain 100X

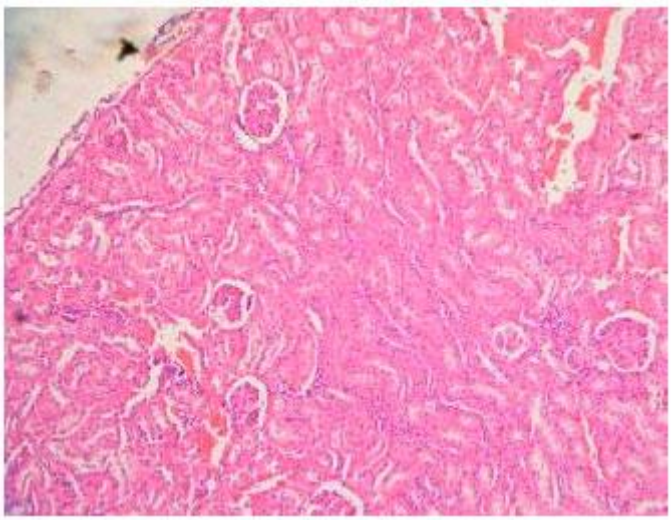
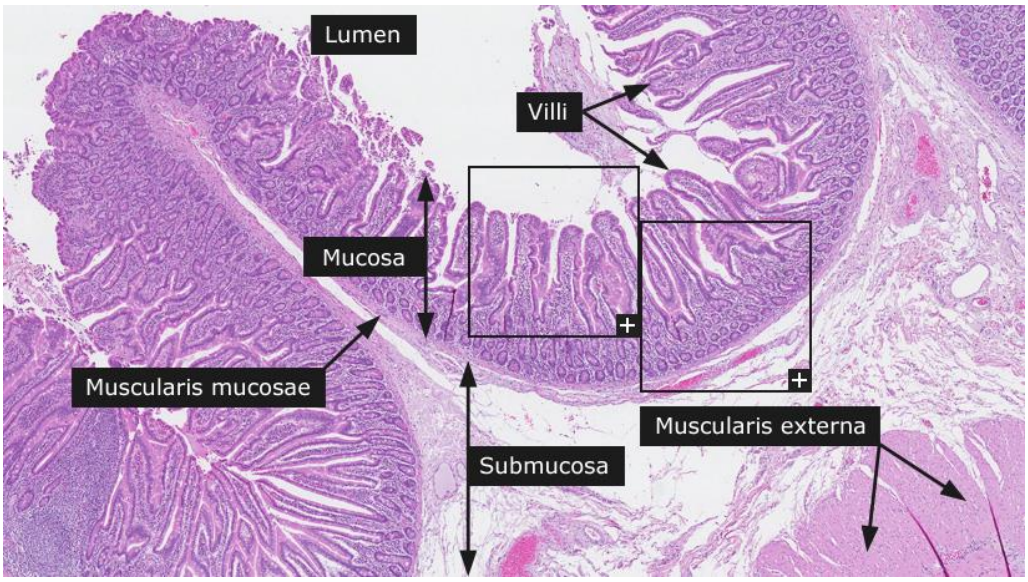


Figure 1.11 Section of kidney from group-IV rat showing congestion, hemorrhages, tubular nephrosis and decrease in glomerular size. H and E stain 100X

1.7.4 Intestine Pathohistological Studies

The animal studies of paraquat intoxification in intestine is limited (Debe *et al.*, 2007; Lalruatfela *et al.*, 2014). Both studies reported histological changes in the intestine followings intra-peritonial administration of paraquat solution at different doeses. According to Lalruatfela *et al.* (2014), the major pathological findings in the rat tissues treated with paraquat were distortion and desquamation of epithelium in the lumen of intestine. Debe *et al.* (2007) revealed mucosal ulceration, loss of villi, luminal and stromal edema, and glandular necrosis in the small intestine. The degree of the abnormality is dose dependent, wherein the degree of distortion increased with the added treatment dose. As compared to the normal histological findings, the distinct characteristics of lumen, villi, mucosa, and muscularis mucosae were depreciated (The-Human-Protein-Atlas, 2020).

A. 

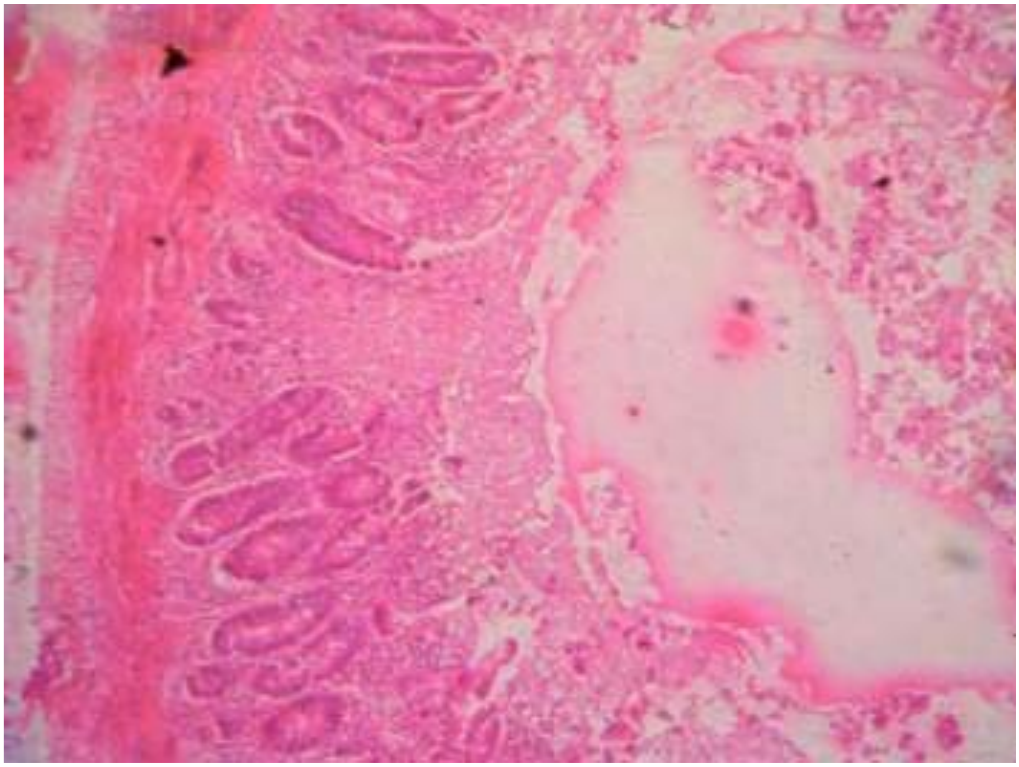
B. 

Figure 1.12 Intestine pathalogical findings in rat treated with PQ

Note:

A. Section of H&E stained intestinal tissue in the normal control rat group; B. sections from intestine of rat showed distortion and desquamation of epithelium in the lumen of intestine after PQ exposure (H&E stain)100X

1.8 Treatment of Paraquat Toxicity

The possibility of recovery clearly depends on the dose of paraquat taken and the time interval between ingestion and the commencement of emergency treatment (WHO, 1984). Treatment should consider both the mechanism of toxicity and toxicokinetics of paraquat poisoning (Table 1.1). Plasma paraquat concentrations, urine and plasma dithionite tests and clinical features provide a good guide to prognosis. Activated charcoal and Fuller's earth are routinely given to minimize further absorption. Elimination methods such as haemodialysis and haemoperfusion are unlikely to change the clinical course. Immunosuppression with dexamethasone, cyclophosphamide and methylprednisolone is widely practiced, but evidence for efficacy is very weak. Antioxidants such as acetylcysteine and salicylate might be beneficial through free radical scavenging, anti-inflammatory and NF-kB inhibitory actions, but their efficacy is yet to be proven clinically (Gawarammana and Buckley, 2011).

Treatment/investigation	Indications	Comment
Decontamination	If within 2–4 h	Use activated charcoal or Fuller's earth
Nasogastric tube	Pharyngeal/oesophageal burns or PQ in urine	Insert prophylactically as early as possible as swallowing becomes difficult later
Urine dithionite test	All patients. If negative, repeat within 24 h	Indicate prognosis. Survival expected if negative test – confirm with plasma paraquat
Plasma paraquat	All patients	Indicate prognosis
EUC, FBC, LFTs, ABG	Repeat at least daily and when clinically indicated	Look for reversible causes. Progressive changes indicate prognosis
Monitor fluid balance	All patients	Declining urine output- correct fluid balance and screen for acute renal failure
Intravenous fluids	Inability to swallow, hypotension	
Haemoperfusion/Haemodialysis	Presentation within 2 h. Acute renal failure WITHOUT pneumonitis	Most likely of use early and in cases with 'borderline exposures'. Futile in very severe or late poisoning
Monitor respiratory rate and oxygen saturation	All patients. AVOID OXYGEN	Look for treatable causes (e.g. infection and pneumothorax). Acute pneumonitis (early) and fibrosis (late) indicate very poor prognosis
Monitor cardiovascular status	All patients	Hypotension not responsive to fluid indicates a very poor prognosis.
Monitor level of consciousness	All patients	If CNS toxicity secondary to hypoxia or acidosis, there is a very poor prognosis
Pain relief and sedation	All patients	Pain relief with opiates and sedation with benzodiazepines as required
Intubation and ventilation	Acute stage – as for any other medical condition	Avoid in acute pneumonitis due to large ingestions and lung fibrosis
Experimental therapy	Consenting patients and clinical trials	No evidence from human clinical trials. Dexamethasone, salicylates and NAC have most support in animal models

Table 1.1 Treatment recommendation following PQ exposure

Source: Gawarammana and Buckley (2011)

1.9 Problem Statement

Residues of PQ in foods are usually detectable especially when it is used as a preharvest desiccant or pre-plant applications in food crops such as cereals where its levels reached 0.2 mg/kg (Akinloye *et al.*, 2011). Paraquat used for weed control pre-planting, new crops pick up traces of the chemical from dead plant debris and soil that it pushes through, thus leading to residues in the harvested crops. For example, small quantities of PQ (< 0.2 ppm) were reported to be detectable in the foliage of certain crops such as sugar beet and cereals. Repeated treatment with PQ resulted in soil build-up and crop damage. Long-term paraquat exposure has been associated with many chronic diseases such as Parkinson's disease, dermatitis, impaired lung function, genotoxicity, and kidney damage in human studies. These studies were correlational studies, and the evidence are weakened by the presence of appropriate normal control to exclude the potential cofounding factors like genetic, lifestyles, and types of exposed toxic substances. An appropriate animal models though could not represent a human body system; this model can provide insight into the effect of mono-herbicide i.e. paraquat on the body organs. Nonetheless, the time required for the development of chronic model is long. The longer the established chronic model, the potentially higher amount of paraquat residues accumulated in the body organs (PAN, 2017; Shin-Yuan Chen, 2010). Thus far, there was study focus on the deposition of PQ in an acute model following intravenous exposure, but not in a long-term PQ exposure models (Masataka *et al.*, 1990).

1.10 Rationale of the Study

Currently available animal model evidence on paraquat toxicity were based on acute toxicity experiments. The evidence of paraquat toxicity based on prolonged paraquat

exposure experiment is limited (Minnema *et al.*, 2014). Nonetheless, unveiling the effect of prolonged paraquat exposure is important as this would provide insight into the impact of prolonged paraquat on the targeted organs, especially the guts and lungs (Claus *et al.*, 2016; Tang *et al.*, 2017b).

1.11 Study Objective

The general objective of the study is to determine the possible effects of three-month paraquat exposure on the morphological features of selected rat major organs.

Specific objectives

i. To compare the tissue morphological change in selected rat organs between control and treatment groups.
ii. To compare the deposition of PQ in selected rat organs between control and treatment groups.

1.12 Overview of the Study

The methodology flowchart of the study shown below

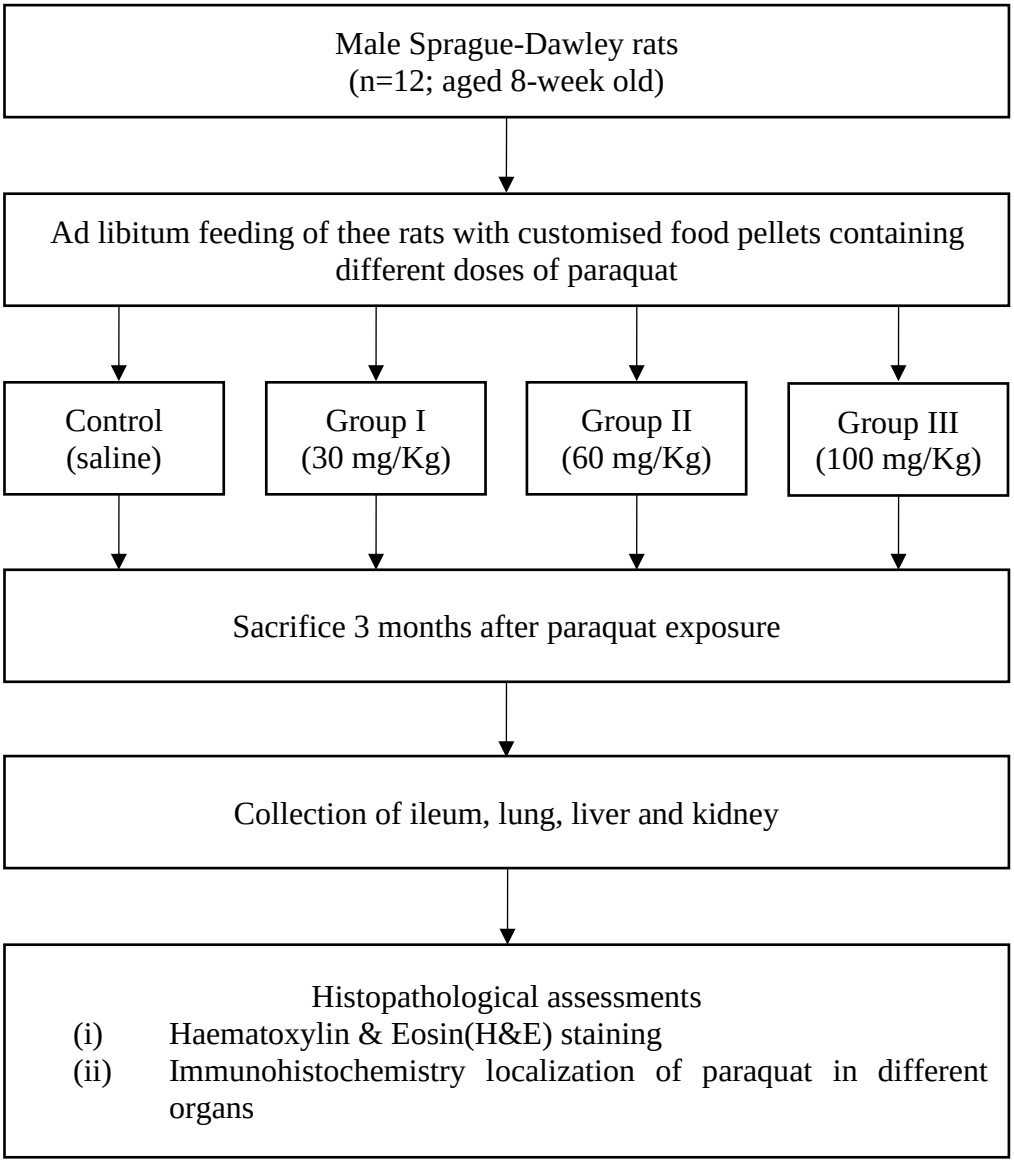


Figure 1.13 Flowchart of study methodology

CHAPTER 2

MATERIAL AND METHOD

2.1 Materials

2.1.1 Rat

Male Sprague-Dawley rats (n=12) aged eight weeks' old were purchased from the Animal Research and Service Center, Health Campus, USM, Kubang Kerian, Kelantan, Malaysia.

2.1.3 List of Chemicals and Reagents

Chemicals used in this study are listed in Table 2.1.

2.1.4 List of Kits and Consumables

Kits and consumables used in this study are listed in Table 2.2.

2.1.5 List of Equipment

Equipment used in this study is listed in Table 2.3.

Table 2.1 List of chemicals

Chemical / Reagent	**Manufacturer**
Food pellet	Sigma Aldrich, USA
Bedding	Sigma Aldrich, USA
Paraquat dichloride (3 bottle of 1L)	Merck, Germany
Absoulute alcohol	Merck, Germany
Xylene	Merck, Germany
Paraffin	Sigma Aldrich, USA
Haematoxylin	Merck, Germany
Eosin	Merck, Germany
10% buffered formaldehyde	Merck, Germany
Formalin	Merck, Germany
Ethanol 70%,90%,100%	Merck, Germany
Sheep anti-Paraquat antibody (ab53431)	Abcam, USA
HRP-conjugated rabbit anti-sheep antibody	Abcam, USA
DPX Mounting Media	Merck, Germany
Phosphate buffered saline tablets	Merck, Germany