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Improvement of kidney histological morphology in nephrotoxic paracetamol-induced rats by *Cassia alata* treatment

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ABSTRACT

Widely used as antipyretics to reduce fever and relieve pain, paracetamol has also been consumed excessively as a means to commit suicide and the incidence is alarming. Paracetamol toxicity as a result of overdose may result in hepatotoxicity and nephrotoxicity. This study was aimed to investigate the therapeutic effects of *Cassia alata* leaf aqueous extract on protecting and improving kidney morphology of rats exposed to an overdose of paracetamol. 25 male Sprague-Dawley rats were divided equally into five groups: (i) negative control group with standard diet; (ii) positive control group induced with paracetamol toxicity at a dose of 3000 mg/kg rat body weight; (iii) paracetamol-induced (3000 mg/kg) followed by *Cassia alata* (200 mg/kg) treatment for 21 days; (iv) 21 days treatment of *Cassia alata* (200 g/kg) followed by paracetamol-induced (3000 mg/kg); and (v) 21 days supplementation of *Cassia alata* extract only. The rats were sacrificed after treatment is completed to harvest the kidney organ for histological examination via hematoxylin and eosin (H&E) staining. The kidney of positive control rats induced with overdosage of paracetamol demonstrated damaged glomeruli, dilated tubules and endothelial rupture in a capsule. All groups treated with *Cassia alata* showed improvement in the histological features of kidney morphology, the size of the glomerulus, and the ratio of normal versus damaged glomerulus compared to paracetamol-induced rats. This study concluded that *Cassia alata* aqueous extract with a dosage of 200 mg/kg are capable to improve kidney morphology damaged by exposure to paracetamol overdose toxicity.

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INTRODUCTION

Paracetamol or acetaminophen is a drug which functions as a pain reliever (non-narcotic analgesics) and widely used to reduce fever (antipyretics) (Rocha *et al.,* 2005). Paracetamol has long been considered a safe drug when consumed properly, but may be dangerous and fatal if taken excessively (Penna and Buchanan, 1991). Paracetamol is the most frequent drug used for self-poisoning in many countries, and its overdose has been frequently reported in teenagers and adults with intentions of self-harm and attempted suicide (Hawton *et al.,* 2013; Rang *et al.,* 2015). Reports have shown that excessive use and an overdose of paracetamol between 200 mg/kg (10 g) to 300mg/kg

(15 g) in adults increases the risk of toxicity resulting in massive hepatic necrosis and eventual fatality (Vermeulen *et al.,* 1992; Larson, 2007).

Although most complication of paracetamol toxicity is associated with liver damage, nephrotoxicity or kidney dysfunction may occur as a secondary effect to hepatoxicity. Rare cases of acute renal tubular necrosis due to paracetamol toxicity, although uncommon, have also been reported (Vermeulen *et al.,* 1992) as well as necrosis in the proximal tubules of the kidney (Hinson *et al.,* 2010). Reports also show that paracetamol overdose resulted in acute renal failure in 1 to 2% of affected patients (Mazer and Perrone, 2008; Bunchorntavakul and Reddy, 2013). Kidney injury due to paracetamol overdose has also been demonstrated in both *in vitro* kidney model and hepatectomized mice (Martin *et al.,* 2016).

At therapeutic dosage, about 5 to 9% of paracetamol undergo oxidation by cytochrome P-450 forming a reactive intermediate metabolite, *N*-acetyl-para-benzo-quinonimine (NAPQI) which are subsequently detoxified through conjugation with glutathione (GSH). Majority of the consumed paracetamol undergo conjugation to sulfated and glucuronidated metabolites before being excreted in the urine (Hollow *et al.,* 1974; Forrest *et al.,* 1982; Larson, 2007; Hodgman and Garrard, 2012). At a toxic dose, excessive intake of paracetamol results in the increased formation of NAPQI and deplete cellular GSH. Presence of excessive NAPQI metabolites will damage cells and macromolecules including mitochondrial proteins (Hinson *et al.,* 2010; Bunchorntavakul and Reddy, 2013). Mitochondrial dysfunction will release an excessive amount of oxidants which in turn will injure cells and tissues leading to necrotic cell death in organs such as the liver and kidney (Du *et al.,* 2016). Mazer and Perrone (2008) reported that NAPQI that can lead to kidney cells destruction primarily renal cortical tubules.

Despite the advancements of modern medicine, the treatment for kidney damage has limited availability. Furthermore, therapy with modern medicine may also pose a risk of adverse events towards patients and are generally more costly compared to traditional medication (Stickel and Schuppan, 2007). The use of natural products as therapeutic medication and antidotes have been documented since ancient civilization. *Cassia alata* is one of the traditional plant being studied due to their high content of flavonoids, alkaloids, carbohydrates, tannins, sterols, saponins, phenols, cardiac glycosides and anthraquinones (El-Mahmood and Doughari, 2008; Hennebelle *et al.,* 2009; Abou Seif, 2016; Adelowo and Oladeji, 2017).

Cassia alata or *Senna alata,* also known as ringworm tree, Christmas candle, candlestick or candle bush (Wuthi-udomlert *et al.,* 2010), is native to Southeast Asia, Northern Australia, Latin America and Africa (Palanichamy and Nagarajan, 1990), and belongs to the *Fabaceae* family and subfamily of *Caesalpinioideae*. *Cassia alata* has been used as alternative medicine to relieve constipation, liver and skin problems (Chatterjee *et al.,* 2012), heart problems, abdominal pain, edema, wound healing, disease of the uterus (Meenupriya *et al.,* 2014), hemorrhoids, blennorrhagia (Sule *et al.,* 2011) and kidney problems (Palanichamy *et al.,* 1988). They have also been shown to possess medicinal properties as an antifungal agent (Chatterjee *et al.,* 2012), antimicrobial, antiparasitic, anti-inflammatory, laxative (Hennebelle *et al.,* 2009), antioxidant (Abu et. al., 2015; Ishak et. al., 2015; Abu et. al., 2016), anthelminthic and as wound healing agent (Meenupriya *et al.,* 2014). These medicinal properties are believed to be the results of their high phytochemical constituents particularly the flavonoid contents. The presence of high phenolics and flavonoids in the aqueous extract of *Cassia alata* are thought to be responsible for its potent antioxidant property (Priyadharshini and Sujatha, 2011). Flavonoids are considered as potent antioxidants as they are capable of effectively scavenging reactive oxygen species (ROS) due to their phenolic hydroxyl groups (Pamulaparthi *et al.,* 2015).

This study was conducted to investigate the effects of *Cassia alata* leaf aqueous extract towards the protection and alleviation of kidney morphology damage due to paracetamol-induced toxicity in an *in vivo* rat model.

MATERIALS AND METHODS

Plant Sampling and Extraction

The leaves of *Cassia alata* plant were collected from Kuala Pilah, Negeri Sembilan, Malaysia. The samples were washed with clean water to remove unwanted dirt, then placed in a 40°C air dryer cabinet for approximately seven days until a constant weight was achieved. The dried leaves were pounded into small particles by mechanical crushing and grounded into a fine powder using laboratory electric blender. To prepare the aqueous extract, 50 g of the resulting powder was dissolved in 500 ml of distilled water to achieve a ratio of 1:10 dilution. The suspension was sieve filtered through several layers of gauze and repeated for several times. The suspension was filtered again using Whatman no. 1 filter paper repeatedly to obtain a clearer purified solution. Finally, the filtrate was sterilized using a syringe filter to attain a sterile and refined aqueous extract of *Cassia alata*. The stock solution was stored in 4°C refrigerator until further use.

Animal Preparation and Treatment Regime

Prior to animal experimentation, ethical approval was obtained from Universiti Kuala Lumpur, Institute of Medical Science Technology (UniKL MESTECH)'s Animal Research Ethics Committee. 25 male Sprague-Dawley rats weighing 300 to 500 gm used in this study were fed with standard rat pellet diet and water ad libitum and housed in standard plastic cages under 12 hours of light and dark cycle. All rats were acclimatized to the environment for seven days prior to the animal experimentation procedure. The rats were divided into five groups with five rats in each group: (1) Negative control group of healthy rats fed ad libitum with water and normal rat pellet for seven days; (2) Positive control group where rats were induced with paracetamol toxicity at a concentration of 3000 mg/kg rat body weight (the paracetamol solution were freshly prepared following the protocol described by Hemabarathy *et al.,* 2009); (3) Rats were given 3000 mg/kg of paracetamol solution followed by 21 days of *Cassia alata* extract at a concentration of 200 mg/kg rat body weight by oral gavage treatment; (4) Rats were fed orally with *Cassia alata* extract (200 mg/kg) pre-treatment for 21 days followed by paracetamol toxicity induction (3000 mg/kg); and (5) Oral gavage supplementation of *Cassia alata* extract only for 21 days. Following completion of treatment regimens, the rats were sacrificed, and the kidneys were harvested for histopathological examination.

Histopathological Examination of Rat Kidney

Rat kidneys were harvested and fixed in 10% buffered formalin to preserve the organ tissue. The tissue specimens were processed using an automated tissue processing machine and embedded in paraffin wax. The blocks were cut at estimated of 5 µm thickness and then subjected to histology staining with hematoxylin and eosin (H&E). Histopathological examinations under the light microscope were conducted to observe morphological differences between the different groups of experimental rats.

Morphometric and Number of Glomerulus

The diameter (µm) of glomerulus for each kidney samples were measured under 20x and 40x light microscopy magnification and the average size for each group were calculated. The sums of normal and damaged glomerulus were also counted under 10 fields of 22 microscopy area at 20x magnification.

RESULTS AND DISCUSSION

Histopathological Examination

In the normal healthy rats (negative control), normal renal tubules (proximal and distal) architecture were observed as displayed in Figure 1 (A). The lumen with the nucleus can also be seen clearly with well-structured cellular boundaries. No shrinkage in size of glomeruli and pathological morphologies were observed in this group.

As shown in Figure 1 (B), all kidney samples of the paracetamol-induced group (positive control) displayed the presence of damaged tissue morphology with dilated renal tubules, severe endothelial rupture of capsule, and shrinkage in size and irregular shape of the glomerulus. This is consistent with a study by Nassar *et al.,* (2010) that reported changes in the cytoarchitecture of the glomeruli, proximal, distal convoluted tubules, and also changes in the cytoarchitecture of interstitium in the histology assessment of nephrotoxicity due to paracetamol overdose.

For paracetamol-induced rats treated with *Cassia alata*, normal morphology in kidney tissues were observed with normal renal tubules (proximal and distal) architecture as exhibited in Figure 1 (C). There was however the presence of mild endothelial ruptures of the capsule in few tissue samples. No shrinkage in size of glomeruli was observed.

As sampled in Figure 1 (D), all kidney tissue of rats pre-treated with *Cassia alata* before paracetamol exposure exhibited normal glomeruli and renal tubules. Similar to the treatment group, there was also the minor presence of endothelial ruptures in the capsule observed in few sample tissues.

In rats supplemented with *Cassia alata* only, normal renal tubules (proximal and distal) architecture were observed across all tissue samples as seen in Figure 1 (E). Regular shapes of glomeruli were observed and no shrinkage in size was seen. Hence, this study confirms that *Cassia alata* are non-toxic to the kidney as there was no evidence of inflammation, necrosis and degeneration in all tissue samples which are consistent with findings by Roy *et al.,* (2016) and Ugbogu *et al.,* (2016).

Glomerulus Morphometric Analysis

Table 1 shows the average diameter (µm) of glomerulus for every experimental group observed under 20x and 40x magnification. The largest average size of glomerulus was recorded in healthy rats (negative control) fed with normal diet (348.4 ± 42.0 µm under 20x magnification; 717.0 ± 91.9 µm under 40x magnification), while the smallest average of glomerulus size was recorded in paracetamol-induced group (318.5 ± 18.1 µm under 20x magnification; 620.3 ± 37.7 µm under 40x magnification). All groups treated with *Cassia alata* recorded a larger average size of glomerulus compared to the paracetamol-induced group which demonstrates the treatment efficacy to improve kidney morphology.

Table 1: Average size of the glomerulus in the kidney of experimental groups

Treatment Group	The average size of glomerulus	
	20x magnification (µm)	40x magnification (µm)
Negative control (standard diet)	348.4 ± 42.0	717.0 ± 91.9
Positive control (paracetamol-induced)	318.5 ± 18.1	620.3 ± 37.7
Paracetamol-induced treated with *Cassia alata*	331.8 ± 29.2	653.5 ± 50.7
Paracetamol-induced pre-treated with *Cassia alata*	326.2 ± 24.8	650.8 ± 68.3
Cassia alata extract only	329.9 ± 28.4	690.8 ± 51.1

Table 2: Number of normal and damaged glomerulus under 10 fields of 22 microscopy area

Treatment Group / Glomerulus morphology	Negative control (standard diet)	Positive control (paracetamol-induced)	Paracetamol-induced treated with *Cassia alata*	Paracetamol-induced pre-treated with *Cassia alata*	*Cassia alata* supplement only
Normal	42	9	34	21	16
Damaged	15	42	26	20	13
Ratio	2.8	0.21	1.31	1.05	1.23

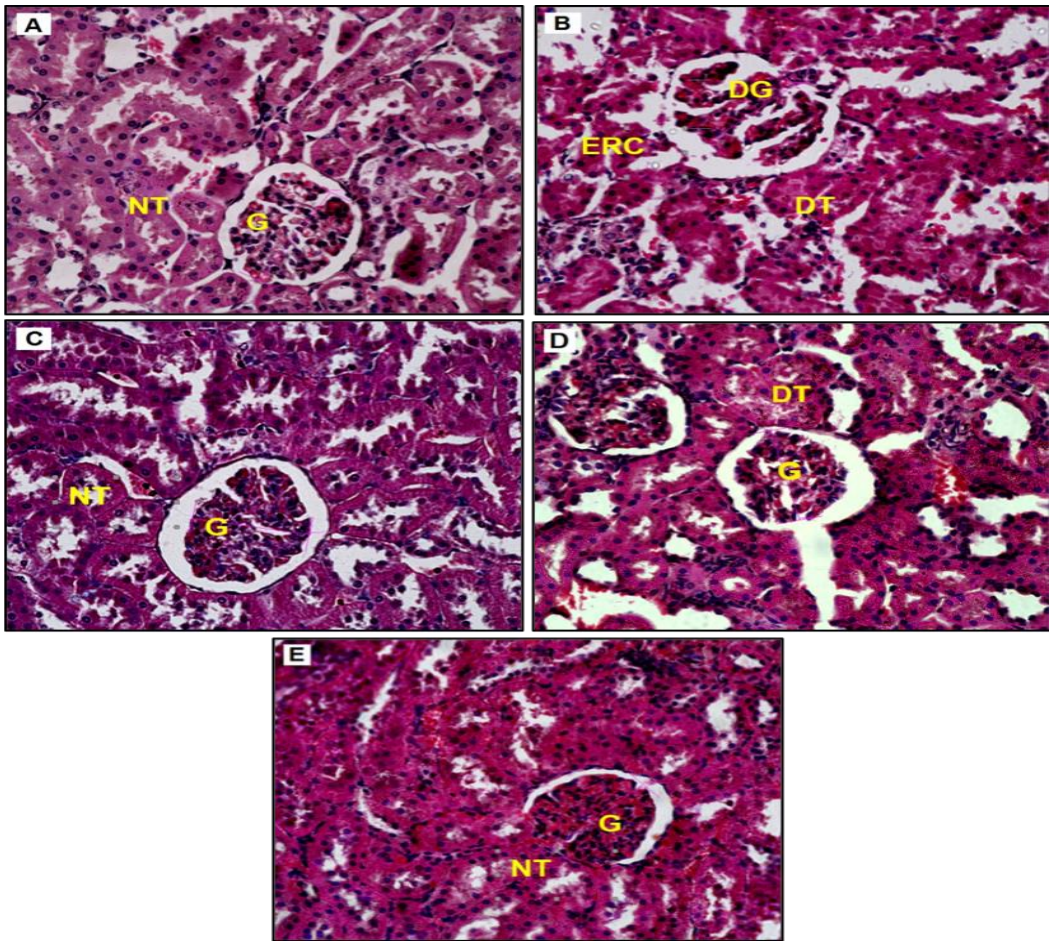

Figure 1: H&E staining of rat kidney under 40x magnification of light microscopy

(A) Negative control (standard diet); normal architectural of the glomerulus (G) and normal renal tubules (NT) with intact cellular boundary. (B) Positive control (paracetamol-induced); damaged glomerulus (DG) with dilated tubules (DT) along with the loss of cellular boundary and endothelial rupture in capsules (ERC). (C) Paracetamol-induced treated with *Cassia alata*; normal glomerulus (G) and renal tubules (NT). (D) Paracetamol-induced pre-treated with *Cassia alata*; glomerulus (G) was normal but the tubules were dilated (DT). (E) *Cassia alata* treatment only; glomerulus (G) and tubules are normal (NT).

Ratio of Normal versus Damaged Glomerulus

Table 2 shows the amount of normal versus damaged glomerulus counted for every experimental group under 20x magnification of light microscopy. In the negative control group, a higher number of the normal glomerulus (42) was observed as compared to the damaged glomerulus (15) for a ratio of 2.8. This follows the trend of morphometric analysis where the negative control group obtained the highest ratio of normal versus damaged glomeruli, while the lowest ratio was observed in the positive control group. Paracetamol-induced rats as the positive control recorded 9 normal glomeruli as opposed to 42 damaged glomeruli with a ratio of 0.21 indicating that paracetamol exposure at 3000 mg/kg induces nephrotoxicity. All groups treated with *Cassia alata* recorded lower ratio compared to the negative control, but higher than the positive control. Paracetamol-induced rats treated with *Cassia alata* obtained 1.31 ratio; pretreatment with *Cassia alata* prior to paracetamol-induced recorded 1.05; while supplementation with *Cassia alata* obtained 1.23 ratio. The higher number of the normal glomerulus in rats supplemented with *Cassia alata* suggests that the plant extract does not exert adverse effects at the given dose of 200 mg/kg as reported in the previous study (Roy *et al.,* 2016). These findings demonstrate the ability of *Cassia alata* to improve glomerulus morphology although not entirely resembling the normal kidney cytoarchitecture of healthy rats.

CONCLUSION

This study demonstrates that damaged kidney morphology induced by paracetamol toxicity can be reversed by *Cassia alata* leaf aqueous extract to a certain extent, but not entirely. All groups treated with *Cassia alata* showed alleviation of damaged renal morphology indicated by the improved histological features, increased size, and the ratio of normal versus damaged glomerulus as compared to paracetamol-induced rats. Findings of this study suggest that *Cassia alata* leaf aqueous extract with a dosage of 200 mg/kg can alleviate kidney damage caused by toxicity of paracetamol exposure and should be further explored.

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REFERENCES

Abou Seif, H. S. 2016. Physiological changes due to hepatotoxicity and the protective role of some medicinal plants. *Beni-Suef University Journal of Basic and Applied Sciences*, 5(2), 134–146.

Abu, I. F., Arifin, A. N., Ambia, K. M. and Ishak, R. 2016. Effects of *Cassia alata* treatment on erythrocyte oxidative stress in hyperglycemic rats. *Science International (Lahore)*, 28(3), 2551-2553.

Abu, I. F., Manoharan, K. B., Ambia, K. M., Noah, R. M. and Ishak, R. 2015. Effects of *Cassia alata* leaf extract towards liver and renal microsomal oxidative stress in hyperglycemic rats. *Bioscience Research*, 12(1), 21-26.

Adelowo, F. and Oladeji, O. 2017. An overview of the phytochemical analysis of bioactive compounds in Senna alata. *Advances in Biochemistry,* 5(5), 102-109.

Bunchorntavakul, C. and Reddy, K. R. 2013. Acetaminophen-related Hepatotoxicity. *Clinics in Liver Disease*, 17(4), 587–607.

Chatterjee, S., Chatterjee, S. and Dutta, S. 2012. An Overview of the ethnophytopathological studies of Cassia alata -an important medicinal plant and the effect of VAM on its growth and productivity. *International Journal of Research in Botany*, 2(4), 13–19.

Du, K., Ramachandran, A. and Jaeschke, H. 2016. Oxidative stress during acetaminophen hepatotoxicity: Sources, pathophysiological role and therapeutic potential. *Redox Biology*, 10, 148–156.

El-Mahmood, A. M. and Doughari, J. H. 2008. Phytochemical screening and antibacterial evaluation of the leaf and root extracts of Cassia alata Linn. *African Journal of Pharmacy and Pharmacology,* 2(7), 124-129.

Forrest, J. A. H., Clements, J. A. and Prescott, L. F. 1982. Clinical Pharmacokinetics of Paracetamol. *Clinical Pharmacokinetics*, 7(2), 93–107.

Hawton, K., Bergen, H., Simkin, S., Dodd, S., Pocock, P., Bernal, W., Gunnell, D. and Kapur, N. 2013. The long-term effect of reduced pack sizes of paracetamol on poisoning deaths and liver transplant activity in England and Wales: interrupted time series analyses. *BMJ*, 346, f403.

Hemabarathy, B., Budin, S. B. and Feizal, V. 2009. Paracetamol hepatotoxicity in rats treated with a crude extract of *Alpinia galanga. Journal of Biological Sciences,* 9(1): 57-62.

Hennebelle, T., Weniger, B., Joseph, H., Sahpaz, S. and Bailleul, F. 2009. Fitoterapia Senna alata. *Fitoterapia*, 80(7), 385–393.

Hinson, J. A., Roberts, D. W. and James, L. P. 2010. Mechanisms of acetaminophen-induced liver necrosis. *Handbook of Experimental Pharmacology,* 196, 369-405.

Hodgman, M. J. and Garrard, A. R. 2012. A Review of acetaminophen poisoning. *Critical Care Clinics*, 28(4), 499–516.

Hollow, D.J., Thorgeirsson, S. S., Potter, W. Z., Hashimoto, M. and Mitchell, J. R. 1974. Acetaminophen-induced hepatic necrosis. VI. Metabolic disposition of toxic and nontoxic doses of acetaminophen. *Pharmacology*, 12(4-5), 251-271.

Ishak, R., Abu, I. F., Lajis, H. M., Ambia, K. M. and Noah, R. M. 2015. Effects of *Cassia alata* treatment towards cardiovascular oxidative stress in hyperglycemic rats. *International Journal of Pharmaceutical Sciences Review and Research*, 34(2), 254-258.

Larson, A. M. 2007. Acetaminophen Hepatotoxicity. *Clinics in Liver Disease*, 11(3), 525–548.

Martin, B., Jimenez-Hernandez, M., Prado, V. and Nogue, S. 2016. Acute kidney injury in paracetamol poisoning. *Medicine Clinica*, 146(5), 233–234.

Mazer, M. and Perrone, J. 2008. Acetaminophen-induced nephrotoxicity: Pathophysiology, clinical manifestations, and management, *Journal of Medical Toxicology,* 4(1), 2–6.

Meenupriya, J., Vinisha, A. and Priya, P. 2014. Cassia alata and Cassia auriculata – Review of their bioactive potential. *World Journal of Pharmaceutical Sciences*, 2(12), 1760–1769.

Nassar, I., Pasupati, T., Judson, J. P. and Segarra, I. 2010. Histopathological study of the hepatic and renal toxicity associated with the co-administration of imatinib and acetaminophen in a preclinical mouse model. *The Malaysian Journal of Pathology*, 32(1), 1–11.

Palanichamy, S. and Nagarajan, S. 1990. Antifungal activity of *Cassia alata* leaf extract. *Journal of Ethnopharmacology*, 29(3), 337–340.

Palanichamy, S., Nagarajan, S. and Devasagayam, M. 1988. Effect of *Cassia alata* leaf extract on hyperglycemic rats. *Journal of Ethnopharmacology*, 22(1), 81–90.

Pamulaparthi, A., Prathap, V. R., Banala, M. and Nanna, R. S. 2015. Total phenolic, flavonoid contents and antioxidant assays in leaf extracts of Senna alata (L.) Roxb. *Journal of Pharmaceutical Sciences and Research*, 8(9), 981–985.

Penna, A. and Buchanan, N. 1991. Paracetamol poisoning in children and hepatotoxicity. *British Journal of Clinical Pharmacology*, 32(2), 143–149.

Priyadharshini, S. D. and Sujatha, V. 2011. Phytochemical investigation of Cassia alata Linn flowers through various in vitro antioxidant assays. *International Journal of Pharmacy and Technology*, 3(4), 3521–3534.

Rang, H. P., Ritter, J. M., Flower, R. J. and Henderson, G. Rang & Dale's Pharmacology, 8th Edn, Churchill Livingstone, 2015.

Rocha, J. B. T., Gabriel, D., Zeni, G., Posser, T., Siqueira, L., Nogueira, C. W., and Folmer, V. 2005. Ebselen and diphenyl diselenide change biochemical hepatic responses to overdosage with paracetamol. *Environmental Toxicology and Pharmacology*, 19(2), 255–261.

Roy, S., Ukil, B., Lyndem, L. M. and El-Nezami, H. 2016. Acute and sub-acute toxicity studies on the effect of Senna alata in Swiss Albino mice. *Cogent Biology*, 2(1).

Stickel, F. and Schuppan, D. 2007. Herbal medicine in the treatment of liver diseases. *Digestive and Liver Disease*, 39(4), 293–304.

Sule, W. F., Okonkwo, I. O., Omo-Ogun, S., Nwanze, J. C., Ojezele, M. O., Ojezele, O. J. and Olaonipekun, T. O. 2011. Phytochemical properties and in-vitro antifungal activity of Senna alata Linn. crude stem bark extract. *Journal of Medicinal Plants Research*, 5(2), 176–183.

Ugbogu, A. E., Okezie, E., Duru, M. and Atasie, O. C. 2016. Toxicity evaluation of the aqueous stem extracts of *Senna alata* in Wistar rats. *American Journal of Biomedical Research*, 4(4), 80–86.

Vermeulen, N. P. E., Bessems, J. G. M., and Van De Straat, R. 1992. Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism-based prevention. *Drug Metabolism Reviews*, 24(3), 367–407.

Wuthi-udomlert, M., Kupittayanant, P. and Gritsanapan, W. 2010. In vitro evaluation of antifungal activity of anthraquinone derivatives of Senna alata. *Journal of Health Research*, 24(3), 117–122.