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Alleviation of renal oxidative stress by *Cassia alata* in acetaminophen-induced nephrotoxic rats

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ABSTRACT

This study was conducted to assess the protective and treatment effects of *Cassia alata* aqueous extract against acetaminophen-induced nephrotoxicity. 25 Sprague-Dawley rats were equally divided into 5 groups; (1) Healthy rats (negative control); (2) Rats induced with acetaminophen toxicity (3000 mg/kg) as the positive pathological control; (3) Rats treated with *Cassia alata* (200 mg/kg, 21 days) and subsequently induced with acetaminophen toxicity (3000 mg/kg); (4) Rats induced with acetaminophen toxicity and subsequently treated with *Cassia alata* (200 mg/kg, 21 days); and (5) Rats supplemented with *Cassia alata* only for 21 days. Following completion of the treatment protocol, rats were sacrificed to harvest kidney organs. Kidney homogenate was subjected to oxidative stress biochemical parameters; malondialdehyde (MDA) content, catalase (CAT) enzyme activity and 1-1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. MDA content in groups treated with *Cassia alata* were all lower compared to the acetaminophen-induced group. Significant differences ($p<0.05$) were observed between acetaminophen-induced rats pre-treated with *Cassia alata* and *Cassia alata* supplementation only compared to positive control. CAT activity for all other *Cassia alata* treatment groups were comparable to healthy rats ($p>0.05$). Total antioxidant DPPH radical scavenging activity was also lowest in the acetaminophen-induced group compared to healthy rats and all *Cassia alata* treatment groups although the results were insignificant ($p>0.05$). Findings of this study suggest that *Cassia alata* aqueous extract (200 mg/kg) possess moderate protective and treatment effects against renal oxidative stress induced by acetaminophen-toxicity.

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INTRODUCTION

Acetaminophen or N-acetyl-para-aminophenol (APAP), more commonly known as paracetamol is a drug with significant analgesic properties and the ability to reduce fever as antipyretics agent (Klinger-Gratz et al., 2018). Widely used globally, acetaminophen's general usage includes to relieve headaches, minor muscle aches, reduce cold and flu, as well as for pain management to relieve post-surgical pain and palliative care actions in cancer patients (Jozwiak-Bebenista and Nowak, 2014). Acetaminophen's basic mechanism of action is believed to involve cyclooxygenase (COX) inhibition with a predominant effect on cyclooxygenase 2

(COX-2) (Ames et al., 1993). Inhibition of COX enzyme in the central nervous system lowers prostaglandin E_2 concentration, which reduces the hypothalamic set-point fever. It also activates the prenominal inhibitory serotonergic pathway in analgesia production (Anderson 2008; Jahr et al., 2010).

Within the therapeutic dose, acetaminophen will be primarily metabolized by sulfation and glucuronidation in the liver (Vermeulen et al., 1992), while five to nine percent will be oxidized by Cytochrome P450 (CYP) enzymes such as CYP2E1, CYP1A2 and CYP3A4, converting it to a highly reactive metabolite known as N-acetyl-p-benzoquinone imine (NAPQI) (Corcoran et al., 1980; Dahlin et al., 1984). Detoxification of NAPQI occurs mainly by glutathione (GSH) conjugation. When taken in overdose, conversion of acetaminophen to NAPQ1 is elevated, while GSH is depleted. Excessive production of NAPQI will react on important protein and macromolecules causing toxicity manifestation such as liver and renal dysfunction (Coles et al., 1988; Mitchell et al., 1973; Rogers et al., 1997; Kaplowitz, 2004).

Even though acetaminophen is categorized as a safe drug, it has been reported as one of the main causal factors of acute liver failure in the United States (U.S) (Lee, 2004; Larson et al., 2005). Intake above the upper limit of acetaminophen's recommended dosage can lead to elevation of serum aminotransferase in healthy adults (Watkins et al., 2006; Harrill et al., 2009). Gooch (2007) has reported that long-term consumption of acetaminophen at a high-dose therapy in more than 10,000 elderly individuals is associated with a significant increase of chronic kidney disease progression. Moreover, renal dysfunction associated with acetaminophen toxicity may also result in high blood pressure, electrolyte imbalances, salt and water retention (Ejaz, 2004). Although the mechanism is unclear, the pathophysiology of renal dysfunction by acetaminophen toxicity has been attributed to CYP-mixed function oxidase isoenzymes present in the kidney. Other mechanisms have also been hypothesized which include the role of prostaglandins and N-deacetylase enzymes (Mazer et al., 2008).

Medicinal plants have currently received global recognition due to their potency, relatively inexpensive and considered generally safe (Sule et al., 2011). Even in developed countries like the U.S and Europe, traditional phytomedicine is continuously in use either for preventing or treating various diseases (Zige et al., 2014). *Cassia alata* or also known as *Senna alata* is a herbaceous medicinal plant that have long been used globally for its benefits in treating various conditions. Belonging to the family of *Fabaceae* or *Leguminosae* (bean family), this plant can be found in Malaysia, Nigeria, Thailand, Australia and tropical America (Zige et al., 2014). It has been traditionally used for treatments of non-infectious diseases like anaemia, constipation, dyspepsia, uterine disorders, convulsion, heart failure, urinary stone, oedema and abdominal pain. It has also been shown to inhibit leukemic cells, possesses antimicrobial properties against bacteria, fungi, protozoa and virus, as well as skin diseases, eczema, gonorrhoea and snakebites. Several parts of the plant have also been used or identified as anti-helminthic, laxative, expectorant, diuretics, purgative, antidiabetic, analgesic and anti-inflammatory agents (Farnsworth and Bunyapraphatsara, 1992; Moriyama et al., 2003; Fernand et al., 2008; Makinde et al., 2007; Wuthiudomlert et al., 2010; Abu et al., 2015; Ishak et al., 2015; Abu et al., 2016; Sugumar et al., 2016).

The phytochemical constituents in *Cassia alata* that are responsible for the medicinal properties include tannins, phenols, saponins, steroids, alkaloids, flavonoids, carbohydrates, anthraquinones, kaempferol and terpenes (Moriyama et al., 2003; Akinyemi et al., 2005; Owoyale et al., 2005; Sule et al., 2011). These chemical constituents, particularly in the leaves, also result in its significant antioxidant properties (Subramaniam and Venugopal, 2001). Due to their potency as an antioxidant, this study was aimed to evaluate the effects of *Cassia alata* on protecting and alleviating renal oxidative stress in acetaminophen-induced experimental rats.

METHODS

Cassia alata Leaves Collection and Extraction

Cassia alata leaves were sampled from Kuala Pilah, Negeri Sembilan, Malaysia. Upon collection, the plant leaves were washed with clean water to remove all unwanted particles. The leaves were then air dried in a cupboard at 40°C temperature for seven days until a constant weight was obtained. Using mechanical crushing apparatus, the dried leaves were pounded and grounded to form a fine powder. 50 g of the powder was soaked and mixed well in 500 ml of sterile distilled water to make up a 1:10 dilution ratio solution. The resulting suspension was filtered through several layers of gauze for up to five times to obtain a clear suspension. The solution was then filtered using Whatman no. 1 filter paper repeatedly to produce a more refined solution, and subsequently subjected to syringe filtration to obtain a pure, sterile *Cassia alata* aqueous extract stock.

Animal Experimentation and Treatment

Following animal ethics approval by Universiti Kuala Lumpur, Institute of Medical Science Technology (UniKL MESTECH)'s Animal Ethics Committee, 25 male Sprague-Dawley rats (300~480 g) were obtained and acclimatized for seven days in standard cages under a controlled environment. The rats were divided equally into five groups and fed with standard rat pellet and water ad libitium. Group one serves as the negative control consisting of healthy rats fed with normal diet. Group two serves as the positive control where the rats were induced with a pathological condition of acetaminophen toxicity. Acetaminophen was dissolved in distilled water at a ratio of 1:2 following the protocol by Hemabarathy et al. (2009), and subsequently fed into rats by oral gavage at a dose of 3000 mg/kg of rat body weight. Rats in group three were pre-treated with 200 mg/kg *Cassia alata* extract daily for 21 days by oral gavage, followed by acetaminophen-induced (3000 mg/kg) toxicity. Rats in group four were induced with acetaminophen toxicity (3000 mg/kg) on day one, followed by *Cassia alata* (200 mg/kg) treatment for 21 days. Group five were fed with *Cassia alata* extract only by oral gavage daily for 21 days. Following completion of treatment regimens, the rats were sacrificed, and kidneys were harvested for oxidative stress biochemical analysis.

Preparation of Kidney Homogenate

The renal homogenates were prepared based on the procedure described by Noori et al. (2009). The renal tissues were minced and homogenized in 0.1 M Tris buffer, pH 7.4 at 1:10 weight per volume in a chilled condition (4°C). The resulting homogenate was centrifuged at 600 x *g* for one hour at -80°C to obtain the supernatant for use in spectrophotometric biochemical assays.

Lipid Peroxidation Assay (MDA Content)

The extent of lipid peroxidation was determined according to the method described by Ledwozyw et al. (1986). This procedure is based on the principle that MDA which is the product of lipid peroxidation reacts with thiobarbituric acid (TBA) producing a pink-coloured product measured spectrophotometrically at a maximum absorbance wavelength of 535 nm. The results were expressed as mean±SEM nmol/mg/protein.

CAT Enzyme Assay

Catalase (CAT) enzyme activity was estimated by adopting the procedure described by Sinha (1972). The principle of this method is based on the dichromate reduction in acetic acid to chromic acetate upon heating in the presence of hydrogen peroxide (H_2O_2). The resulting formation of a green product was measured calorimetrically at 570 nm. This protocol measures H_2O_2 concentration expressed as mean±SEM µg/mL which is inversely proportional to CAT enzyme activity. The lower the remaining H_2O_2 measured indicates the higher CAT enzyme activity present in the solution.

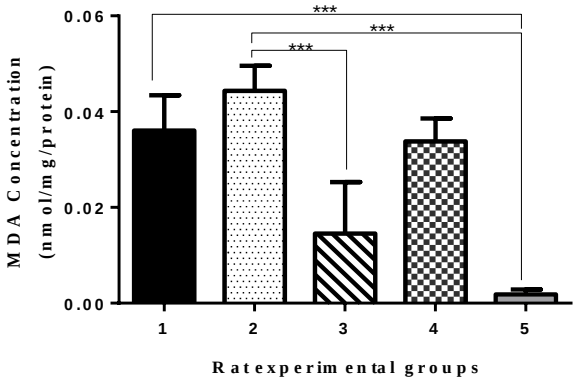
Total Antioxidant (DPPH Radical Scavenging) Assay

The total antioxidant assay protocol described by Blois (1958) was based on the ability of samples to scavenge stable free 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. This assay results in the decolourization formation of purple-coloured DPPH solution which indicates the ability of electron donation of samples when there is an addition of a radical species or antioxidant. The absorbance was measured spectrophotometrically at 517 nm. The results were expressed as mean±SEM % of DPPH radical scavenging activity.

RESULTS

MDA Content

Figure 1 shows that MDA content was highest in the positive control group (0.044±0.005 nmol/mg/protein) where acetaminophen exposure was expected to increase lipid peroxidation. The lowest MDA content was recorded by rat group supplemented with *Cassia alata* only (0.002±0.001 nmol/mg/protein). Rat group pretreated with *Cassia alata* prior to acetaminophen exposure managed to significantly lower MDA content (0.015±0.011 nmol/mg/protein) compared to the positive control group ($p<0.05$). Rats treated with *Cassia alata* following acetaminophen exposure also had lower MDA content (0.034±0.005 nmol/mg/protein) compared to positive control group and comparable ($p>0.05$) to healthy rats (0.036±0.007 nmol/mg/protein).

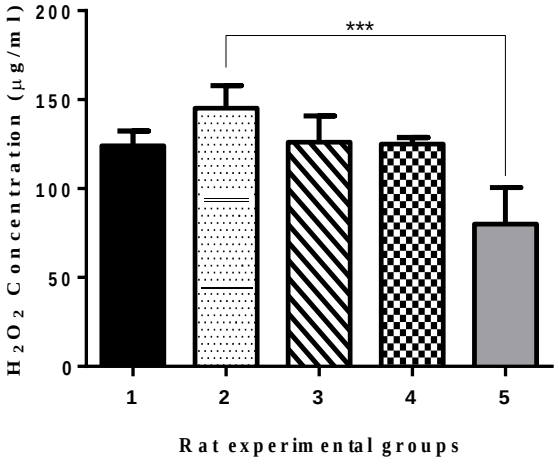


Legend:
***p<0.05, significantly different
1: Negative control
2: Acetaminophen-induced
3: *Cassia alata* pre-treatment + acetaminophen-induced
4: Acetaminophen-induced + *Cassia alata* treatment
5: *Cassia alata* only

Figure 1: MDA Concentration in Rat Kidney

CAT Enzyme Activity

Figure 2 shows the H_2O_2 concentration present in the measured renal homogenate indicating CAT enzyme activity whereby the lower concentration of H_2O_2 indicates higher CAT enzyme activity. The highest CAT enzyme activity was recorded in rat group supplemented with *Cassia alata* only (80.0±20.5 µg/ml H_2O_2) while expectedly, the pathological group of acetaminophen-induced rats recorded the lowest CAT activity (145.2±12.6 µg/ml); both groups significantly different ($p<0.05$). Rats pre-treated with *Cassia alata* prior to toxicity exposure and rats treated with *Cassia alata* following toxicity recorded 126±14.9 µg/ml and 125±3.83 µg/ml H_2O_2 respectively, both comparable ($p>0.05$) to healthy rat group (124±8.49 µg/ml H_2O_2).

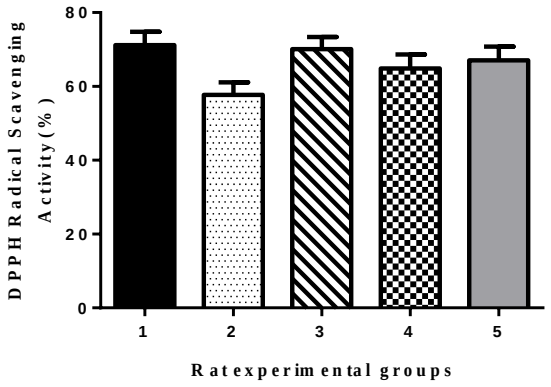


Legend:
***p<0.05, significantly different
1: Negative control
2: Acetaminophen-induced
3: *Cassia alata* pre-treatment + acetaminophen-induced
4: Acetaminophen-induced + *Cassia alata* treatment
5: *Cassia alata* only

Figure 2: H_2O_2 concentration (inversely proportional to CAT activity) in rat kidney

DPPH Radical Scavenging Activity

Figure 3 shows the percentage of DPPH radical scavenging activity as an indicator of total antioxidant activity in renal homogenate. As expected, rat group induced with acetaminophen exposure recorded the lowest total antioxidant activity at 57.7±3.41 % while healthy group recorded 71.2±3.63 %. All treatment groups with *Cassia alata* recorded higher total antioxidant activities compared to the acetaminophen-induced group but lower than the healthy group although no significant differences were observed across all groups.



Legend:
1: Negative control
2: Acetaminophen-induced
3: *Cassia alata* pre-treatment + acetaminophen-induced
4: Acetaminophen-induced + *Cassia alata* treatment
5: *Cassia alata* only

Figure 3: Total antioxidant (DPPH-radical scavenging) activity in rat kidney

DISCUSSION

In oxidative stress condition which occurs in acetaminophen toxicity, oxidants and free radicals are elevated whereas antioxidant levels are depleted. The imbalance between reactive oxygen species and antioxidants may increase lipid peroxidation activity producing end products such as MDA (Hassanin et al., 2015). In acetaminophen toxicity, excessive NAPQI will bind covalently to protein sulfhydryl groups on target organs such as the liver and kidney resulting in necrotic cellular damage. Decreasing level of GSH antioxidant observed in acetaminophen toxicity further impair the condition as GSH is responsible to conjugate NAPQI for its detoxification and elimination (Hinson et al., 2010). This study clearly shows that administration of acetaminophen at a concentration of 3000 mg/kg induces a toxicity condition which increases lipid peroxidation activity in the kidney as indicated by the high MDA content. All groups treated with *Cassia alata* managed to lower MDA content particularly in the pre-treatment and *Cassia alata* supplementation groups suggesting that the extracted content managed to reduce lipid peroxidation in oxidative stress condition as does in normal physiological state. *Cassia alata* treatment also managed to reverse the lipid peroxidation activity in acetaminophen-induced rats to normal condition as the result is comparable to healthy rats.

Presence of endogenous antioxidant enzymes is paramount in oxidative stress condition. CAT enzyme is one of the important endogenous antioxidant responsible for decomposing reactive oxygen species such as H_2O_2 into safer contents which are water and oxygen (Sies, 2017). The results of CAT enzyme activity in this study correspond to MDA content in rat kidney. The highest activity was ob-

served in *Cassia alata treatment* group which is significantly lower than the acetaminophen toxicity group, whereas all other treatment groups were comparable to healthy rats. Although the results were less significant, the trend shows that *Cassia alata* managed to boost or enhance endogenous enzyme activity such as CAT to overcome oxidative stress to a certain extent. This is supported by total antioxidant activity assessed by DPPH radical scavenging properties. Similar to CAT, the differences across all groups are not significant, but the trend shows that total antioxidant activities were higher in all treatment groups than the acetaminophen-induced group and are comparable to healthy rats.

CONCLUSION

Findings of this study suggest that *Cassia alata* at a dosage of 200 mg/kg possess moderate protective and treatment effects against renal oxidative stress induced by an overdose of acetaminophen. More significant results might be obtained with a higher concentration of *Cassia alata* extract as this study only assessed a single concentration at 200 mg/kg. Treatment of *Cassia alata* extract in healthy rats were also able to reduce MDA content and increase CAT enzyme activity compared to the negative control suggesting its supplementation benefit to maintain and improve oxidative stress condition. These antioxidative properties of *Cassia alata* are attributed to its excellent phytochemical profile which contains a vast amount of beneficial chemical constituents such as flavonoids, tannins and polyphenols which are able to boost antioxidant networks to prevent or reverse cellular damage caused by lipid peroxidation in an oxidative stress environment.

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