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ABSTRACT

Background: Injury to the liver resulting from drug exposure is known as hepatotoxicity. Drug-induced liver damage is a clinical consequence that can be challenging to recognise, avoid, and manage. After administering *Centella asiatica* extract to rats that have hepatotoxicity induced by AICl₃ and D-galactose, we evaluated the histopathological alterations in the hepatic tissue and measured the concentration of oxidative and anti-oxidative activity.

Objective: The objective of this study is to investigate the protective benefits of *Centella asiatica* extract on AlCl₃ and D-galactose-induced hepatotoxicity.

Materials and methods: Male albino Wistar rats were used in this study. *Centella asiatica* extract (100mg, 200mg and 300mg/kg/day) was given orally to the $AlCl_3$ and D-gal induced hepatotoxic rats for seventy days. At the end of treatment, the liver was harvested and the activity levels of oxidative and antioxidative enzymes were determined. Histopathological changes in the liver were also documented.

Results: *Centella asiatica* extract significantly raised the levels of SOD and catalase in liver homogenates, while lowering MDA levels and suppressing histopathological changes such as bridging necrosis, intralobular degeneration, focal necrosis, and fibrosis alterations in the liver.

Conclusion: Centella asiatica extract reduced oxidative stress levels in drug-induced hepatotoxicity by attenuating histological changes and normalising the levels of oxidative stress indicators in the liver.

KEYWORDS: Centella asiatica, Hepatotoxicity, AlCl₃, D-galactose, Oxidative stress.

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INTRODUCTION

The liver is an important organ, necessary for metabolic and detoxifying processes as well as for the preservation of the body's homeostasis. The liver is the primary site of detoxification in the body, where harmful substances are metabolised and eliminated. It has an intricate web of enzymes, transporters, and metabolic pathways which transforms toxins into less dangerous forms that can be easily eliminated. However, this complexity of the liver makes it more vulnerable to injury during the process of detoxification, when the body is overworked or when there are extremely toxic substances present in the body [1,2].

Liver damage or dysfunction is brought on by excessive drug or xenobiotic usage and is referred to as hepatotoxicity or liver toxicity. Hepatotoxins, often referred to as hepato-toxicants, are substances that can potentially damage the liver. These substances include natural and industrial chemicals, overdoses of pharmaceutical substances, industrial pollutants and dietary supplements [3] Several drugs can damage the liver even when they are consumed within therapeutic limits. In addition to the direct injury caused by toxic substances, hepatotoxicity can result from immune system-mediated reactions, reactive metabolites produced in the body, and direct damage from toxic chemicals. These factors can impact hepatocytes, biliary epithelial cells, and the liver architecture [4].

Exposure to aluminium chloride $(AlCl_3)$ can be dangerous to the liver. Aluminium enters the human body through cosmetics, diet, pesticides, and air pollution [5].

Oxidative phosphorylation, which involves the electron transport chain (ETC), is the process by which mitochondria produce adenosine triphosphate (ATP). Elevated oxidative stress alters the ETC and prevent the synthesis of ATP resulting in mitochondrial energy metabolism disorder, a factor that can cause liver injury. The changes in the ETC pathway during injury to the liver is an important potential mechanism for liver injury [6].

D-galactosamine (D-gal), a hepatotoxicant, which is widely present in macroalgae, plants and dairy waste is also used in the production *Int J Anat Res 2023, 11(4):8740-47.* ISSN 2321-4287

of low-calorie sweeteners [7]. D-gal has shown to cause acute hepatic necrosis and cirrhosis in rats during long-term administration. Its toxicity results from the reduction of uridine pools, affecting hepatocellular function and cell membrane porousness, leading to cell death. It also causes cholestasis due to its damaging effect on bile ducts [8].

Centella asiatica (CA) is also referred to as Indian pennywort (EN), Gotu kola (SL), Brahmi (Hindi), Mandukaparni (Ayurvedic), buak bok (Thai), kaki kuda (Indonesia), Yuhong-Yuhong (Philippines), and Pegaga (Malaysia). It belongs to the Apiaceae family of the plant kingdom and has been proclaimed as a cauldron containing many phytochemicals which have potentially protective functions. The triterpene saponosides are the dominant phytochemicals present in this plant [9]. For many years, the use of CA has been recognised as a treatment for a wide range of illnesses, including eczema, asthma, stomach ulcers, gastrointestinal and neurodegenerative disorders [10]. Because of its many health benefits, including its ability to act as an antioxidant, an anti-inflammatory, its wound healing properties, and capacity to improve memory, the usage of CA in food and beverage products has grown over time [11]. Information on the protective impact of CA extracts on hepatoxicity has not been available. In this study, we postulate that CA can recover and preserve the activity levels of oxidative liver enzymes and maintain the structural integrity of the liver in the face of hepatoxic insult. This study specifically looked at the effects of a CA extract on body weight changes, oxidative stress measurements in the liver tissue, and histological changes after hepatoxicity induced by $AICI_3$ and D-gal.

MATERIALS AND METHODS

Animals: Thirty albino males Wistar rats (220-250g) were obtained from the Perniagaan Usaha Cahaya in Selangor, Malaysia, when they were around 3 months old. In a temperature controlled setting with 12-hour alternative cycles, the rats were housed in the rats were housed in cages with two animals per cage at University Putra Malaysia (UPM), Malaysia. The animals were given unlimited water *ad libitum* and regular food pellets (Harlan, UK). The Insti-

tutional Animal Care and Use Committee (IACUC) issued the ethics approval certificate UPM/ IACUC/AUP-R071/2020.All experiments were carried out in accordance with the IACUC and UPM procedures.

Plant extract and Chemicals: D-gal and AlCl₃ were acquired from Sigma Aldrich, USA, while the CA extract was obtained from Universiti Teknologi MARA (UiTM), Selangor, Malaysia. Analytical grades of chemicals were utilized in the experiment. For intraperitoneal (i.p.) injection, D-gal was mixed in distilled water; for oral delivery, distilled water was used to dissolve AlCl₃ and CA extract.

Experimental Design: Following one week of acclimatisation, thirty male albino Wistar rats were randomly chosen to five separate groups, with 6 rats in each group. The groups were the control group (normal saline (i.p.) and distilled water (oral)), the model group (AICl₂ 200 mg/kg/orally/day + D-gal 60 mg/kg/i.p./day), the CA 100 group (AlCl₃ 200 mg/kg/orally/day + D-gal 60 mg/kg/i.p./day + CA 100 mg/kg/orally/ day), the CA 200 group (AICl, 200 mg/kg/orally/ day + D-gal 60 mg/kg/i.p./day + CA 200 mg/kg/ orally/day), and the CA 300 group (AICl, 200 mg/ kg/orally/day + D-gal 60 mg/kg/i.p./day + CA 100 mg/kg/orally/day). The rats were given seventy days of treatment before being decapitated to end their lives. Decapitation was proven to be the most advantageous type of euthanasia for preventing chemicals such as gases and anaesthetics from polluting liver tissues.

Body weight measurement: Body weight of the rats was measured in first week of the experiment and in the last week of the experiment (on the day of sacrifice).

Sample Collection and Preparation: Following the euthanasia of the rats, the liver tissues were extracted and immersed in ice-cold saline. After being rinsed in phosphate buffer saline (PBS), the hepatic tissues were maintained at -80 °C for biochemical examination. The liver tissues for histological studies were cleaned with normal saline and immediately deposited in a 10% formalin solution for a week. The liver sample was then processed to examine histological changes.

Histopathological Changes of the Liver: The livers harvested from the rats that were preserved in 10% formalin were washed in a *Int J Anat Res 2023, 11(4):8740-47.* ISSN 2321-4287

phosphate buffer solution. Following that, it underwent processing and was encased in paraffin wax. Following tissue sectioning at a thicknessµof 5 m using a microtome (Rotary Microtome RF-600), the tissues were stained with hematoxylin and eosin (H&E). Manual System Microscope (Olympus, BX43) was used to study the histopathological changes. Images were depicted at a magnification of 40X using an image analyser (Nikon H500L). Histological alterations for sinusoidal hyperaemia, inflammation, and dead cells were observed in the portal region.

Measurement of lipid peroxidation and antioxidant enzymes: Malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) levels in the rat liver homogenates were estimated as part of the lipid peroxidation (LPO) assessment. LPO produces MDA as a byproduct. Using the thiobarbituric acid (TBA) reagent, the MDA levels were assessed in accordance with the protocol described by Ohkawa et al [12]. The measurements yielded quantifiable data, which were reported as imol of MDA produced per milligram of protein.

In further assessment of antioxidant enzyme activities in the liver homogenates of rats involved measuring the levels of SOD. The evaluation followed the methodology described by Misra and Fridovich [13]. The amount of SOD present was measured and shown in units per milligram of protein per minute. Additionally, CAT levels of activity were measured by applying a method described by Maehly and Chance. [14]. These levels were communicated as imol of hydrogen peroxide (H2O2) metabolized per milligram of protein per minute.

Statistical Analysis: Mean \pm standard deviation (SD) (n=6) was used to express the values. After one-way ANOVA, Tukey's post hoc test was used to analyse the data. Differences in p values less than 0.05 were considered statistically significant when comparing. GraphPad Pris (version 9) was the software program used for all the analysis.

RESULTS

Impact of CA extract on bodyweight on hepatotoxicity induced by D-gal and $AICl_3$. The initial weight measurement before start of the experiment showed that all the rats did not

show any significant weight difference among the groups. However, on the last day of the experiment, it was observed that the body weights of the rats given $AlCl_3 + D$ -gal were considerably lower than those of the normal control rats. Additionally, in comparison to the rats exposed to $AlCl_3 + D$ -gal, the rats treated with CA at doses of 200 and 300 mg/kg bw showed no significant reduction of body weight. (Figure 1).



Fig. 1: It depicts the first and final days of the experimental rats' experiment. *p 0.05 compared to control and $AICI_3 + D$ -gal; #p 0.05 compared to $AICI_3 + D$ -gal + CA treatment. The data is shown as mean S.D., n = 6.

Impact of CA extract on hepatotoxicity induced by AICl₃ and D-gal on liver LPO, SOD, and Catalase: Malondialdehyde (MDA), a byproduct of lipid peroxidation, was found in much higher concentrations in the livers of rats treated with AlCl₂+D-gal than in the livers of control rats. However, when AICl₂+ D-gal-treated rats were co-administered with CA at doses of 100, 200 and 300 mg/kg bw, the MDA levels in their livers were significantly decreased when compared to AlCl₂+ D-gal- exposed rats, as shown in (Figure 2A). Additionally, the levels of antioxidant enzymes like superoxide dismutase (SOD) have also been observed to be significantly lower in the livers of rats treated with AlCl₃+D-gal. However, while these rats had been co-administered with CA at doses of 100, 200, 300 mg/kg bw, the SOD levels in their livers were significantly increased as compared to those in AlCl₂+D-gal induced rats (Figure 2B). Furthermore, another antioxidant enzyme catalase (CAT) levels were also observed to be significantly decreased in the livers of rats treated with AlCl₂+D-gal. However, while rats were co-administered with CA at doses of 100, 200, and 300 mg/kg bw, the CAT levels of their livers had increased as compared to those in AlCl₃+D-galexposed rats (Figure 2C).



Fig. 2: Represents the oxidative stress and antioxidant enzymes in the liver of experimental rats. (A) Lipid Peroxidation (LPO); (B) Superoxidase dismutase (SOD); (C) Catalase (CAT). Data are expressed as mean \pm S.D, n = 6, *p < 0.05 versus control and AlCl_a + D-gal; #p < 0.05 versus AlCl_a + D-gal + CA treatment.



Fig. 3: Represents the histopathological changes in the liver of experimental rats. Scale bar = 50 im and ×40 eye-piece magnification. CV (Central vein), DC (Dead cell).

Impact of CA extract on AICl₃ and D-gal induced hepatotoxicity in liver histopathology: In normal control rats, the liver histology reveals well-structured lobules, distinct hepatocytes, regular sinusoids, and a central vein at the lobule's center. In contrast, rats exposed to AICl₃+D-gal have liver tissues with showed increased eosinophilic (pink) staining, which signifies damaged or dead cells. On the other hand, treatment with 100 and 200 mg/kg bw CA AlCl,+D-gal induced rats, results in considerable improvement, yet there were some cellular alterations which remained. However, the liver histology in AlCl₂+D-gal rats treated with 300 mg/kg bw CA are almost identical to the control rats (Figure 3).

DISCUSSION

Triterpenoids or saponins, glycosides, flavonoids, alkaloids, certain vitamins, and are among the phytochemicals found in CA. [15]. The therapeutic benefits of CA, including its anti-inflammatory, wound-healing, memoryenhancing, and antioxidant properties, have been the subject of intense research in recent years. The potential of CA as a plant-based alternative natural antioxidant, particularly in protecting the brain's antioxidant defense system against aging-related alterations, has grown significantly in the last several years [16]. In our experimental work, a decrease in body weight serves as a marker for the deterioration of the general health status of rats. According to reports, AICl, may cause biochemical dysfunction and toxicological effects that pose a major risk to human health [17]. The oxidative damage produced by D-gal has been well documented and is the main cause for its hepatotoxicity [18]. It has been observed in this study that rats in the AlCl₃ + D-gal treated group had a significantly lower total body weight than rats in the AlCl, + D-gal and CA treated groups as well as the controls, respectively. The increase in body weight of the rats was in proportion to an increased dosage of CA.

The results of this study show that exposure to excessive amounts of AlCl₃ has altered body weight. This change is most likely due to the inhibition of protein synthesis in the liver, decrease in feed intake, and/or malabsorption of nutrients brought on by the effects of AlCl₃ on the gastrointestinal tract [19, 20] The decreased food intake seen in the AlCl₃-treated group could be explained by exposure to AlCl₃. Therefore, our results were in line with those reported by El-Demerdash and Zhu et al. [21,22] who observed that rats' absolute and relative liver weights, as well as their overall body weight, were dramatically disturbed by high AlCl₃ exposure. Despite the lack of prior research on D-gal's direct impact on lab rat body weight, we believe that D-gal's capacity to induce inflammatory, hepatorenal, and mitochondrial dysfunction may have played a role in the weight loss [23].

Many caffeic acid derivatives and flavonols, including quercetin and kaempferol [24], catechin, rutin, and naringin [22], have been shown to be present in CA. Several of these compounds have been demonstrated to be strong antioxidants. Because of its antioxidant qualities, the co-administration of CA reduced the in vivo effects of AlCl₃+D-gal. These findings suggested that CA, most likely because of its ability to scavenge radicals, might help reduce the toxicity of AlCl₃+D-gal. Hence, body weight is corrected with CA therapy.

Malondialdehyde (MDA), the byproduct of lipid peroxidation, is typically used to quantify the amount of lipid peroxidation (LPO) present in liver homogenates. The MDA levels in the liver tissue of the $AlCl_3$ -+ D-gal treated group were greater than those in the control group and the $AlCl_3$ + D-gal + CA treated group. This may be because of increased reactive Fe⁺² caused by biological membrane peroxidation [25,26].

The transferrin protein that carries Fe⁺³ can bind aluminum because the majority of Al⁺³ ionic radicals are like Fe⁺³ radicals, which causes an accumulation of Fe⁺² in cells, according to studies by Wu et al [26] and Newairy et al [27]. Furthermore, it has been documented that the inactivation of enzymes involved in antioxidant systems, such as SOD, and CAT activities, may result in a rise in MDA concentration [21,25]. The accumulation of AlCl₂ in the liver altered the homeostasis of copper and zinc, in turn reducing their capacity to bind to antioxidant enzymes, thus leading to the malfunctioning of these enzymes. MDA levels rise as a result of oxidative damage and the known inflammatory effects of aluminum, in addition to the antioxidant blocking effects of aluminum accumulation. MDA levels in livers of rats in the $AlCl_3$ + D-gal + CA treated groups showed reduction of MDA, suggesting the potential antioxidant benefits of CA.

In comparison to other plant components, leaves of CA exhibited the highest antioxidant *Int J Anat Res 2023, 11(4):8740-47.* ISSN 2321-4287

activity and the has largest phenolic content, according to Zainol et al. [24].

This finding implies that the main source of CA antioxidative properties are its phenolic chemicals. On the other hand, ethanolic extract from the root of CA showed the highest activity, according to Abdul-Hamid et al. [28], even though it did not differ significantly from the leaves. The antioxidative action of several phytochemical components of CA may be attributed to the decrease of hydroperoxides, the inactivation of free radicals, the chelation of metal ions, or a combination of these mechanisms. CA is an excellent hydroxyl and superoxide radical scavenger as well as an inhibitor of lipid peroxidation [29].

The findings revealed alterations in the liver tissue's SOD and CAT activity, which suggested an oxidative injury to the liver. In this experiment, the rats exposed to $AlCl_3$ + D-gal showed a considerable decrease in their levels of the enzymes SOD and CAT when compared to the control group. On the other hand, CA 300 therapy led to a notable rise in the level of activity of antioxidant enzymes (SOD and CAT).

An imbalance between the generation of reactive oxygen species and the antioxidative pathway led to oxidative stress which favors free radical formation. The present investigation demonstrated that the antioxidant defense system was unable to counteract the influx of reactive oxygen species produced by exposure to AICl₂+ D-gal due to a considerable decrease in the liver's antioxidant enzyme activity (CAT and SOD). Supplementing the rats with CA has a shown to have preventative effect on the breakdown of the antioxidant defense system. Our findings concur with information from related rat research [30,31]. According to this study, CA may have antioxidant capabilities because of its ability to scavenge free radicals and inhibit lipid peroxidation. Accordingly, earlier research demonstrating the capacity of CA to scavenge DPPH free radicals has shown a significant level of antioxidant activity which could be attributed to the chemicals that are found in the CA that are phenolic and flavonoids [32]. In this study significant increases in mass

periportal ± bridging necrosis, intralobular degeneration and focal necrosis, and fibrosis were detected in the AlCl₃ + D-gal treated group. In contrast, these periportal ± bridging necrosis was decreased by CA coadministration. Additionally, CA markedly reduced localized necrosis and intralobular degeneration. Rats administered 300 mg/kg of CA showed a significant reduction in liver fibrosis. The results of this investigation demonstrated CA ability to prevent liver damage brought on by AlCl₃ + D-gal. Previous research has demonstrated the protective benefits of CA on livers injured by various medications in several additional investigations [30,33,34]. Furthermore, it has been demonstrated that madecassoside, another triterpenoid found in CA, prevents cell death after it is exposed to oxidative stress caused by H₂O₂. As a result, CA may shield cells from oxidative damage [35].

CONCLUSION

This work clearly demonstrates that oxidative stress produced by $AICI_3$ -+ D-gal has a great influence on the oxidant and antioxidant system and the alteration of which leads to cellular level damage in liver. This imbalance is lessened by the co-administration of CA, which is evident in this study on rats treated with AlCl₃ + D-gal, wherein CA has a mitigating effect on oxidative stress because of its scavenging activity of free radicals. To precisely identify the mechanism or mechanisms of action of CA on oxidative stress markers in rats intoxicated with AlCl₃ + D-gal, more investigation is necessary. In the future, CA may prove to be a highly promising herb for the treatment of drug-induced hepatotoxicity.

ORCiD

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Author Contributions

T V: Designed and performed the experiments. Analysed the data and wrote the manuscript.

P K G: Analysed the data and assisted in manuscript preparation.

B K: Statistical, images analysis and manuscript preparation.

S J: Performed the experiments and reviewed the manuscript.

M T H B B: Design of the study, guidance in manuscript preparation

W T: Reviewed the manuscript and language editing.

M A M M: Designed and conceptualised the study, reviewed the results, and prepare the manuscript for final submission.

Conflicts of Interests: None

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