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Evaluation of anti-hyperuricemic effects of *Alocasia longiloba* Miq. (Keladi Candik) extracts in potassium oxonate induced rat model

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ABSTRACT

Hyperuricemia has become a significant public-health concern in recent years, and the available treatments have been reported to have an adverse side effect on patients. *Alocasia longiloba* has been used traditionally in Malaysia for treating gout, inflammation, and wounds. However, the plant has not been investigated for its effects on hyperuricemia. This study investigated the anti-hyperuricemic and anti-inflammatory effects of *A. longiloba* extracts in hyperuricemic rats induced by potassium oxonate (250 mg/kg body weight). Rats were given *A. longiloba* extracts or a standard drug for two-week, and blood and tissue samples were collected for analysis. Results show that *A. longiloba* extracts significantly reduced serum uric acid levels in hyperuricemic rats and inhibited xanthine oxidase (XOD) activity in the liver and kidney, which could be the mechanism underlying the urate-lowering effects. The extracts also significantly ($p < 0.05$) reduced the levels of proinflammatory cytokines (IL-18 and IL-1β) in serum samples and had hepatoprotective and nephroprotective effects in hyperuricemic rats. The study supports the use of *A. longiloba* as a complementary therapy for treating hyperuricemia.

1. Introduction

Hyperuricemia (HU), is a metabolic disorder characterized by abnormally increased levels of uric acid (UA) in the human blood exceeding 6 mg/dL in women and 7 mg/dL in men [1–3]. Studies have reported that the prevalence of HU has increased significantly in recent years due to changes in lifestyle such as dietary pattern and consumption of excess alcohols drinks [4,5]. In Asia, the prevalence of HU was estimated in the range of 15.4% to 26.1% [6–8]. In addition, several epidemiological studies have shown that HU is associated with increased risk of developing various diseases such as hypertension, gout, cardiovascular disease, and chronic kidney disease [9,10]. UA is a waste product of purine metabolism and its production is mainly facilitated by the enzyme called xanthine oxidase (XOD). XOD is primarily found in the liver, and is a key enzyme that involve in the UA synthesis pathway in the human body by

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catalyzing the oxidation of hypoxanthine to xanthine and subsequently to UA [11–13].

Available treatments to manage HU have been focused on inhibiting the UA synthesis using XOD inhibitors and excretion promoter drugs, such as allopurinol, colchicine, steroids and non-steroidal anti-inflammatory [14]. Studies have revealed that inhibition of XOD can reduce the formation of UA or increase the kidney's ability to eliminate UA from the body. However, these drugs have shown several adverse side effects [15]. Therefore, there is a need to develop alternative or complementary therapy [16,17].

One of the proposed approaches in overcoming HU is herbal medicine which is the use of natural product that reduce UA concentration and prevent the occurrence of HU [18,19]. A classic example for herbal medicine for HU treatment is the usage of medicinal plants or plant-based products. Plant-based medicine, therefore prevents HU by inhibiting XOD and reduce the formation of UA [19,20]. Over the past few years, a significant work has been done on medicinal plants for discovering new herbal drugs that can be used in combating various diseases [21–24]. Increased consumption of plant-derived products or functional foods can prevent or reduce the risk for diseases, possibly due to the fact that plants contain secondary metabolites that are known to have therapeutic effects [25,26].

In this study, *A. longiloba* plant was selected on the basis of its tradition medicinal values. *A. longiloba*, locally known as keladi candik, is a medicinal plant which has been used for treating wound and inflammation by the local communities in the state of Kelantan, north-eastern corner of peninsula Malaysia [27–29]. Traditionally, the juice from the fruit and petiole parts of *A. longiloba* have been primarily used. The preparation includes grinding the petiole for applying onto wounded skin and also boiling of the fruit to be drank [27]. In some cases, the plant has been reported to treat cough and fever [29]. Furthermore, the rhizome paste is used as a poultice to treat furuncle. Our previous study showed that *A. longiloba* extracts possess major phytochemical compounds (i.e., alkaloids, phenolic, terpenoids and saponins) which are known to have high medicinal values like antioxidant, anti-inflammatory and anti-hyperuricemic activities [30]. The traditional uses of this plant suggest it possesses anti-hyperuricemic and anti-inflammatory effects. To our knowledge, the anti-hyperuricemia activity of this plant has not been reported. Hence, the present study investigates the anti-hyperuricemic and anti-inflammatory effects of *A. longiloba* extract using well-established animal model.

2. Methods

2.1. Chemicals and apparatus

All the chemicals, reagents and assay kits used for *in vivo* study were of analytical and biological grade purchased from Abcam (Abcam, Cambridge, United Kingdom) and Sigma Aldrich (St Louis, Missouri, USA): Potassium oxonate (PO), commercial kit used for determining uric acid, xanthine oxidase (XOD) Assay and enzyme-linked immunosorbent assay (ELISA), were obtained from Abcam. Ethanol 95% and carboxy methyl cellulose sodium (CMC-Na) were purchased from Sigma. Animal diet and bedding were purchased from Animal Research and Service Centre (ARASC), Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.

2.2. Plant collection

A. longiloba was collected from plants growing in a natural population in Kota Bahru, Kelantan, Malaysia (6.1211° N, 102.3178° E) by Mr. Ferid Abdulhafiz. The plant was authenticated by Dr. Zulhazman Hamzah, Faculty of Earth science, Universiti Malaysia Kelantan. The voucher and herbarium samples were deposited in Herbarium of the Faculty of Earth Science, Universiti Malaysia Kelantan, Jeli, Kelantan, Malaysia (Voucher number: UMK00288).

2.3. Preparation of plant extract

The plant tissues (fruit and petiole) were carefully separated from the mother plant and washed under running water. The tissues then dried under the oven for 72 h at 40 °C. The dried plant materials were grounded into a fine powder using electrical blender (Milux MFP-9625 heavy duty blender). The powdered samples were subjected to soxhlet extraction according to Ref. [31], with the solid to solvent ratio of 1:10 (1 g of sample to 10 mL of solvent). After the extraction, the solvent was evaporated under vacuum evaporator and crude extract was kept in the freezer until further use.

2.4. Animals

The adult male Sprague Dawley rats (six-week-old, weighing 180 ± 10 g) were obtained from ARASC. Animals were used according to the suggested ethical guidelines for the care of laboratory animals [32], and all experimental procedures used in the present study were approved by Institutional Animal Care and Use Committee (IACUC) of Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Kota Bahru, Malaysia (Approval number: UMK/FPV/ACUC/PG/1/2020). The rats were randomly selected, labelled and kept in the polycarbonate rat cage (410 × 282 × 153 mm) for one week to adapt to the laboratory conditions before any experimental manipulation. The rats were housed in an animal room (Department of Paraclinical Science, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan) with an air-conditioned environment at 22 to 25 °C in 12-h light, 12-h dark condition (6:00 a.m.–6:00 p.m.) and humidity at the range of 60 to 65%. Animals accessed to standard laboratory feed and sterile tap water *ad libitum* until used for experiments, and the cages and water bottles were cleaned once every three days. All efforts were made to reduce pain, distress and suffering of the experimental animals.

2.5. Establishment of hyperuricemic rat model and drug administration

In the present study, the uricase inhibitor potassium-oxonate was administered to induce experimental model of hyperuricemia in rats according to previous reports [33–35], with modifications. Potassium oxonate, extracts and standard drug (allopurinol, as a positive control) were dissolved in carboxy methyl cellulose sodium solution (0.5% CMC-Na). All doses were expressed in mg per kg body weight (mg/kg b.wt) of the respective drug or extracts. The volume of drug or extract administered were based on body weight measured immediately before each dose. Rats were divided into seven groups (n = 5): group I (normal, 0.5% CMC-Na), group II (PO + 0.5% CMC-Na, served as vehicle control), group III (PO + allopurinol at 5 mg/kg, served as positive control), group IV (PO + fruit extract at 250 mg/kg), group V (PO + fruit extract at 500 mg/kg), group VI (PO + petiole extract at 250 mg/kg), and group VII (PO + petiole extract at 500 mg/kg). The rats were administered with PO (250 mg/kg) or CMC-Na (normal) intraperitoneally at 9:00 a.m. for 14 consecutive days. The water, allopurinol and plant extracts were administered orally at 10:00 a.m. They were administered also for 14 consecutive days. Thereafter, all rats were allowed to access to water and commercial chow *ad libitum* throughout the period of observation. All the PO, 0.5% CMC-Na and plant extracts were prepared freshly. The doses administered in this study were selected based on the established toxicity study, which supported the safe usage of the extracts within the chosen dosage range.

2.6. Blood and tissue sample collection

All rats were fasted for 15-h prior to post-mortem examination. The rats were euthanized using carbon dioxide inhalation followed by cervical dislocation. Blood sample was collected from the posterior vena cava into clean ethylenediaminetetraacetic acid (EDTA) bottles from each necropsied rat. The blood sample was allowed to clot approximately 1 h at room temperature and then centrifuged at 10,000×*g* for 15 min to obtain the serum. The serum sample was stored at −20 °C until assayed. Concurrently, the liver tissue was rapidly and carefully separated on ice-plate, according to the method described previously Mo et al. (2007). The liver tissue then homogenized in 9 vol of 50 mM ice-cold potassium phosphate buffer (pH 7.8) containing 5 mM ethylenediaminetetraacetic acid disodium salt (EDTA2Na) and 1 mM phenylmethanesulfonyl fluoride. The homogenate was centrifuged at 3000 rpm at 4 °C for 10 min and the supernatant was used for further experiments.

2.7. Determination of serum uric acid levels

The serum UA levels after 14-day PO and extracts administration were measured to determine the therapeutic effects of *A. longiloba* extracts by enzymatic colorimetric method using a uric acid assay (ab65344) kit (Abcam, Cambridge, United Kingdom). The protocol was in accordance of kit manufacturer's instruction.

2.8. Measurement of serum of xanthine oxidase activity

The serum and liver xanthine oxidase activities were determined by enzymatic colorimetric, method using a Xanthine Oxidase Assay (ab102522) kit (Abcam, Cambridge, United Kingdom). The assay protocol was in accordance of kit manufacturer's instruction.

2.9. Measurement of pro-inflammatory cytokines levels

The levels of inflammatory cytokines (IL-18 and IL-1β) in the serum in PO induced hyperuricemic rats were measured using the enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, United Kingdom). The detection method was performed according to the instructions of manufacturer.

2.10. Histopathological analysis

Histopathological study was conducted on organ samples of the kidneys and liver. The tissues were processed according to previous report [36]. Organs were harvested and fixed in 10% buffered formalin for 48-h and then formalin-fixed tissues were sliced to 0.6 cm and arranged in cassette. The tissues were then further processed using automatic Dip and dunk tissue processors (Leica TP1020, Buffalo Grove, United States) for 15 h followed by paraffin embedding, trimming and sectioning. About 4 μm thickness tissue section were obtained on a rotary microtome (Leica RM2245, Buffalo Grove, United States). The tissue sections were then mounted on a microscope slide and placed on an oven at 42 °C and allowed to dry overnight. Consequently, the material was stained with hematoxylin and eosin stain to detect any abnormality lesions. Qualitative assessment was carried out to observe histologic alterations in PO-induced and normal rats. The histological examination of the samples was conducted using a compound light microscope (Olympus CX-21, Tokyo, Japan).

2.11. Statistical analysis

All data were expressed as the mean ± standard error of the mean (S.E.M.). The data were analyzed by one-way analysis of variance (ANOVA) and means were compared by Duncan multiple range test (DMRT) at $p < 0.05$ using SPSS v. 21.0 statistical software program. All data were presented as mean ± standard error. Letters (i.e. a, b, c, d) were used to indicate statistical differences between means.

3. Results

3.1. A. longiloba extracts decreased the level of UA in PO-induced hyperuricemic rats

The plant extracts at 250 and 500 mg/kg were orally given for 14-day on PO-induced hyperuricemic rats and we evaluated the serum UA by the uric acid assay as described in the above section. As shown in Fig. 1, the serum UA level in the PO-induced hyperuricemic rats (model group, II) was significantly higher than that of the healthy control (I) group ($p < 0.05$). By contrast, the allopurinol (positive control) group (III) demonstrated significantly lower levels of serum UA ($p < 0.05$). The fruit and petiole extracts-treated groups (IV-VII) demonstrated decreased serum UA levels at low (250 mg/kg) and high (500 mg/kg) dosages compared with the hyperuricemic control. Interestingly both plant extracts treatments had almost similar UA-reductive effect to that of allopurinol and no significant differences ($p < 0.05$) were found between the effects of allopurinol and plant extracts treatment.

3.2. A. longiloba extracts inhibited XOD activity in PO-induced hyperuricemic rats

XOD in the liver and serum induces UA production. Thus, to study the mechanism of the inhibitory effect of *A. longiloba* extracts on HU, the XOD activity in the liver and serum was evaluated. The results revealed that, XOD activity was significantly ($p < 0.05$) increased in the PO group (II) compared to healthy control (I) group (Figs. 2 and 3). Treatment with *A. longiloba* fruit extract at the doses of 250 and 500 mg/kg was able to inhibit liver XOD activity by 11.18% and 16.44%, respectively (IV–V). The petiole extract treatment at the dose of 250 and 500 mg/kg was able to inhibit 6.08% and 16.01% of XOD activity (VI-VII). The XOD inhibitor, allopurinol (ALL) treated group (III) significantly inhibited XOD activity by 18.62% in the liver. The results indicated that the inhibitory effect of allopurinol on XOD activity was significantly higher than the fruit and petiole extracts, even at the highest dosage of both extracts ($P < 0.05$).

The results revealed that, XOD activity in serum was significantly ($p < 0.05$) increased in the PO model group (II) compared with the control group (I). The serum XOD activity showed that the fruit extract treatment at the doses of 250 and 500 mg/kg (IV–V) was able to inhibit 29.69% and 45.79% of XOD activity, respectively. The petiole extract treatment at the dose of 250 and 500 mg/kg (VI-VII) was able to inhibit 44.07% and 45.08% of XOD activity, whilst allopurinol (ALL) inhibited XOD activity of 55.63% (III).

In this study both *A. longiloba* extracts (fruit and petiole) showed a dose-dependent effects on serum UA levels and liver XOD inhibitory activity (Fig. 4). A linear correlation coefficient analysis was performed to assess the relationship between liver XOD activity and serum UA levels. The result showed a positive correlation between the two variables ($r = 0.8039$, $n = 5$).

3.3. A. longiloba extracts decreased proinflammatory cytokines levels in PO-induced rats

HU triggered the production of pro-inflammatory mediators and induced an inflammatory reaction. The results of this study showed that hyperuricemic rats demonstrated increased serum levels of proinflammatory cytokines (IL-18 and IL-1β) when compared with normal control (I) group (Fig. 5 a&b). *A. longiloba* extracts (fruit and petiole) treatment remarkably downregulated the serum levels of proinflammatory cytokines (IL-18 and IL-1β) in hyperuricemic rats (IV-VII). The positive control drug, allopurinol (group III), also significantly reduced the levels of proinflammatory cytokines compared with the HU control (II).

3.4. Effects of A. longiloba on histopathology changes of PO-induced rats

3.4.1. A. longiloba extracts ameliorates PO-induced kidney lesions

The histopathology examination showed mild to moderate congestion, glomerular atrophy, hemorrhage and infiltration of inflammatory cells in the kidney of PO-injected hyperuricemic rats (Fig. 6). In hyperuricemia model group (Fig. B&C), a mild to

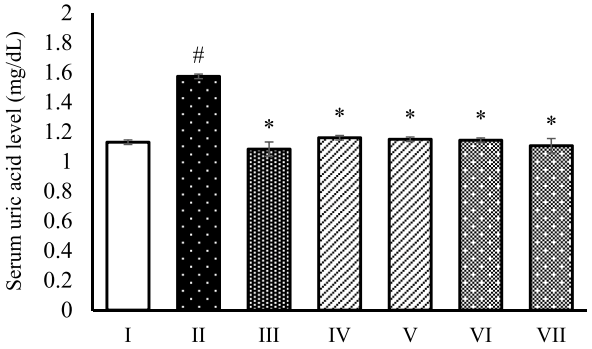

Fig. 1. Effects of *A. longiloba* extracts on serum uric acid levels in PO-induced hyperuricemic rats. Data were analyzed using *t*-test. Treatment groups II - VII given PO (250 mg/kg) for induction of HU; I = Normal rats; II = hyperuricemic model group; III = Standard treatment (allopurinol 5 mg/kg b.wt); IV = EtOH fruit extract (250 mg/kg b.wt), V = EtOH fruit extract (500 mg/kg b.wt), VI = EtOH petiole extract (250 mg/kg b.wt), VII = EtOH petiole extract (500 mg/kg b.wt). B.wt = body weight. (#) $P < 0.05$ versus healthy control group, (*) $P < 0.05$ versus HU control group.

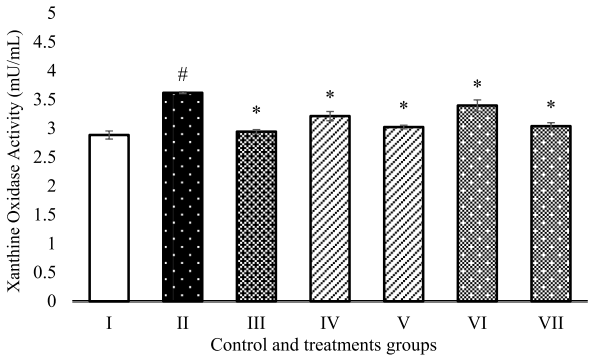

Fig. 2. Effect of *A. longiloba* extracts on XOD activity in rat's liver. Data are presented as mean ± SEM (n = 5), analyzed using *t*-test. Treatment groups II - VII given PO (250 mg/kg) for induction of HU; I = Normal rats; II = hyperuricemic model; III = Standard treatment (allopurinol 5 mg/kg b.wt); IV = EtOH fruit extract (250 mg/kg b.wt), V = EtOH fruit extract (500 mg/kg b.wt), VI = EtOH petiole extract (250 mg/kg b.wt), VII = EtOH petiole extract (500 mg/kg b.wt). B.wt = body weight. (#) $P < 0.05$ versus healthy control group (I), (*) $P < 0.05$ versus HU control group (II).

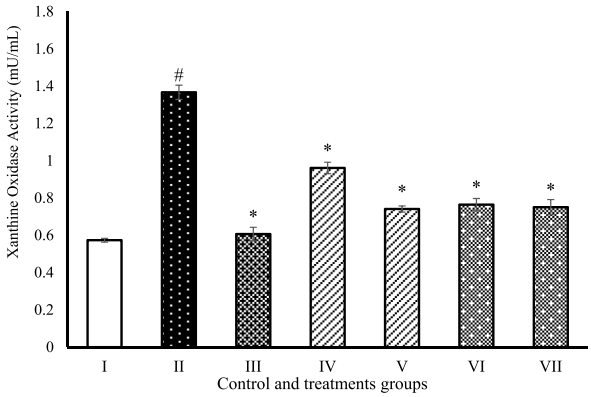

Fig. 3. Effect of *A. longiloba* extracts on serum XOD activity. Data are presented as mean ± SEM (n = 5), and analyzed using *t*-test. Treatment groups II - VII given PO (250 mg/kg) for induction of HU; I = Normal rats; II = hyperuricemic model group; III = Standard treatment (allopurinol 5 mg/kg b.wt); IV = EtOH fruit extract (250 mg/kg b.wt), V = EtOH fruit extract (500 mg/kg b.wt), VI = EtOH petiole extract (250 mg/kg b.wt), VII = EtOH petiole extract (500 mg/kg b.wt). B.wt = body weight. (#) $P < 0.05$ versus healthy control group, (*) $P < 0.05$ versus HU control group.

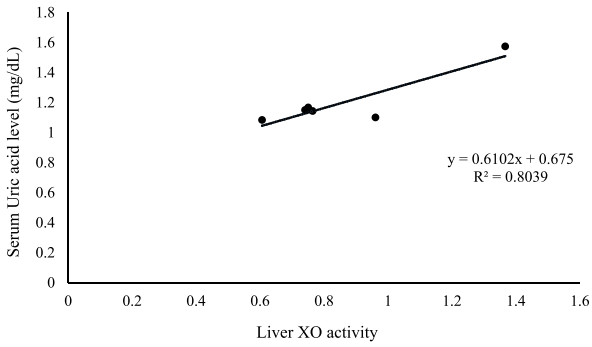

Fig. 4. Correlation between xanthine oxidase activity and serum uric acid levels.

moderate congestion, glomerular atrophy, hemorrhage and infiltration of inflammatory cells were observed. However, fruit and petiole extract effectively ameliorated these renal histopathological changes in hyperuricemic rats (Fig. 6E–H). In addition, allopurinol treated groups significantly improved the renal histopathological changes (Fig. 6D). In this study, no histopathological changes were observed in control group (Fig. A).

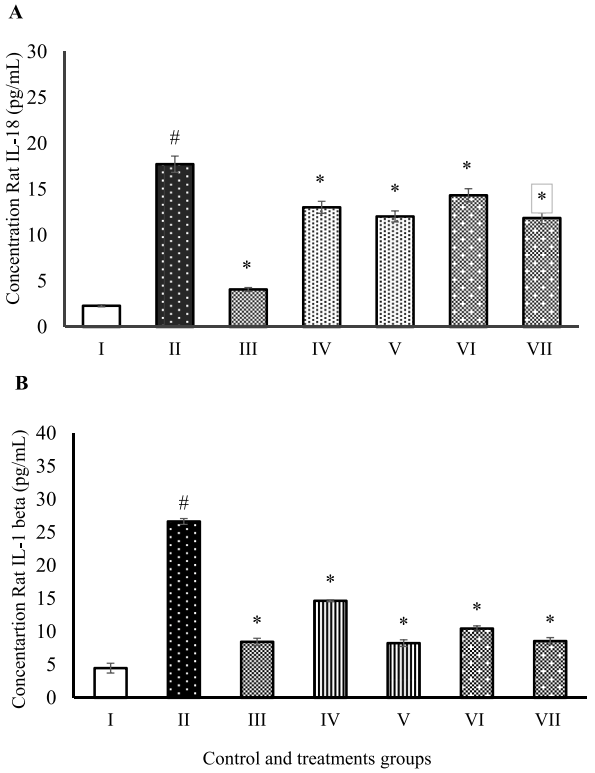

Fig. 5. Effects of *A. longiloba* extracts on the levels of proinflammatory cytokines; IL-18 (a) and IL-1β B (b) in serum. Data are showed as mean ± S.E. M of 5 rats in each group. Treatment groups II - VII given PO (250 mg/kg) for induction of hypeuricemia; I = Normal rats; II = hyperuricemic model; III = Standard treatment (allopurinol 5 mg/kg b.wt); IV = fruit extract (250 mg/kg b.wt), V = fruit extract (500 mg/kg b.wt), VI = petiole extract (250 mg/kg b.wt), VII = petiole extract (500 mg/kg b.wt). ([a]) $P < 0.05$ significantly different compared to control group (I), ([b]) $P < 0.05$ versus HU control group and were significantly different (ANOVA, *t*-test).

3.4.2. A. longiloba extracts ameliorates PO-induced liver lesions

Liver histopathology showed that PO injection caused pathological changes in the form of mild congestion and hemorrhage in the liver organ (Fig. B). However, the liver lesions were ameliorated in the plant extracts treatment groups (Fig. 7D–G). The histological architecture of liver sections of normal rats shows normal cellular hepatic architecture with distinct hepatic cells and sinusoidal space (Fig. A). Similarly, the histopathological morphology in the allopurinol treated group were normal (Fig. C).

4. Discussion

Alocasia longiloba Miq has been widely used as traditional herbal medicine against gout and wound in north-eastern corner of peninsula Malaysia, it's *in vitro* anti-hyperuricemia and wound healing activities were reported in our previous studies [27,28,30]. However, anti-hyperuricemia and anti-inflammatory effects in animal model and its mechanism of action have not been reported. In the current study, we demonstrated that the fruit and petiole extracts of *A. longiloba* significantly reduced serum UA levels and XOD activity (serum and liver) in PO-induced hyperuricemic rats. In addition, *A. longiloba* extracts treatment remarkably downregulated the serum levels of proinflammatory cytokines (IL-1β and IL-18) in hyperuricemic rats. Furthermore, A. *longiloba* extracts exhibited hepatic and nephron-protective effects in hyperuricemic rats.

Hyperuricemia in animals is generated by blocking the enzyme (uricase) that converts uric acid to allantoin with intraperitoneal injection of PO. This model has been used by several researchers to study the anti-hyperuricemia properties of medicinal plants [31–40]. In the present experiment, serum UA levels were significantly elevated after PO administration, indicating the successful establishment of the model. By contrast, the allopurinol-treated group demonstrated significantly lower levels of serum UA ($p < 0.05$). In addition, the fruit and petiole extracts-treated groups demonstrated decreased serum UA levels at low (250 mg/kg) and high (500 mg/kg) dosages compared with the hyperuricemic control.

XOD is the key enzyme that involve in the UA synthesis in the human body by catalyzing the oxidation of hypoxanthine to xanthine and subsequently to UA [38–41]. Therefore, downregulating of XOD activity is one of the most common strategies to lower blood UA levels [42]. XOD inhibitors, such as allopurinol, febuxostat, steroids and non-steroidal anti-inflammatory drugs, are used to treat hyperuricemia. However, these drugs have been reported to have serious adverse side effects [19,43]. In the present study, the fruit and petiole extracts demonstrated significant serum and liver XOD inhibitory activity in hyperuricemic rats, indicating that inhibitory

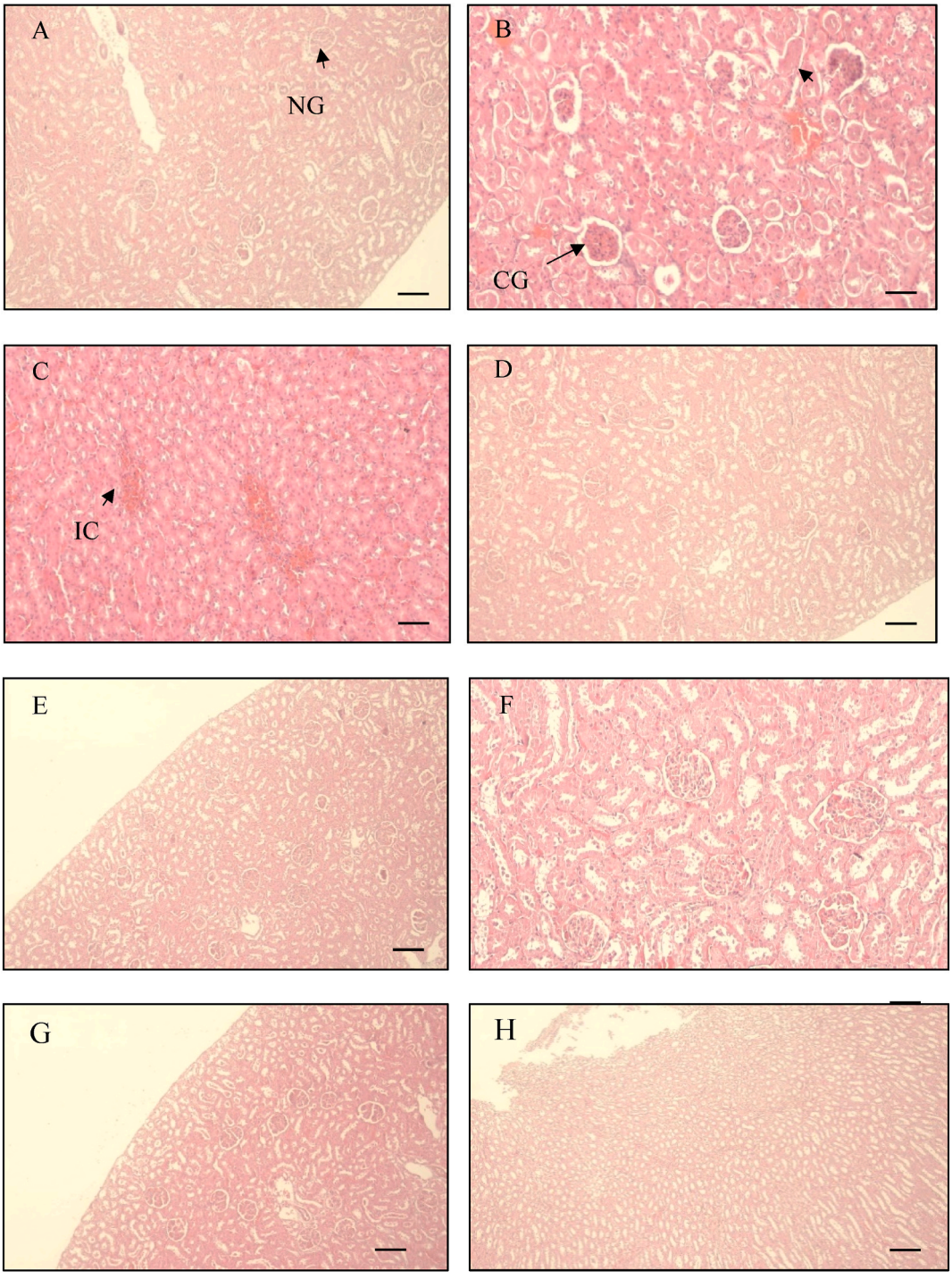

Fig. 6. Effect of *A. longiloba* extracts on kidney histology in PO-induced hyperuricemic rats. (A) Normal group, (B&C) hyperuricemia model group (250 mg/kg b.wt.), (D) allopurinol (5 mg/kg b.wt) + PO, (E) Fruit extract (250 mg/kg b.wt) + PO, (F) Fruit extract (500 mg/kg b.wt) + PO, (G) Petiole extract (250 mg/kg b.wt) + PO, (H) Petiole extract (500 mg/kg b.wt) + PO. All images were captured at 40x (H&E) magnification. NG (normal glomerulus); CG (congested glomerulus); IC (inflammatory cells). Scale bar: 40 μm.

activity towards XOD could be the mechanism of anti-hyperuricemia effects of extracts. Several studies have documented the involvement of XOD in the production of UA [44].

UA has been reported to cause inflammatory response through stimulation of pro-inflammatory mediators. Recent studies have found that HU leads to the production of proinflammatory cytokines including IL-1β and IL-18 [45–47]. Several evidences suggest that

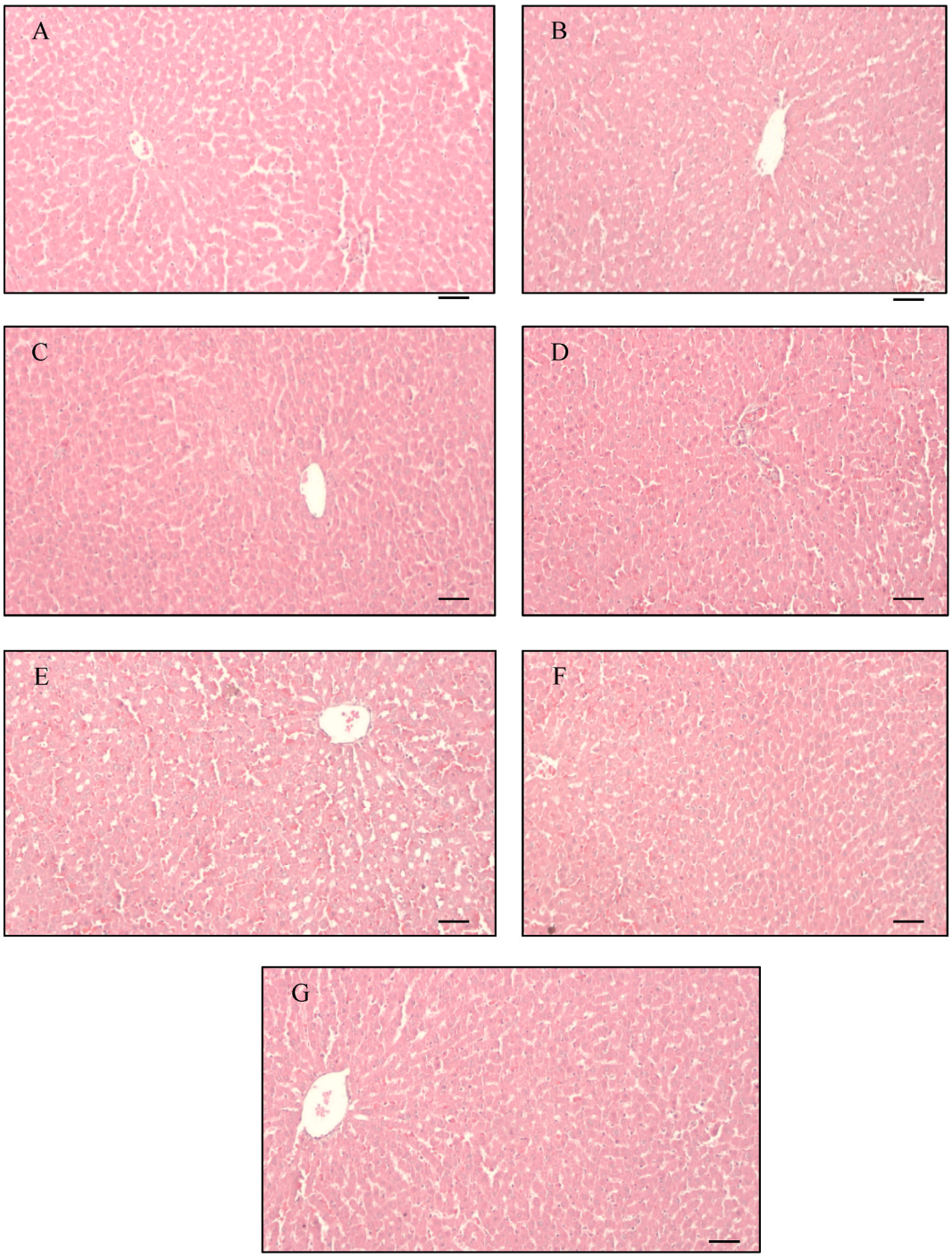

Fig. 7. Effect of *A. longiloba* extracts on liver histology in PO-induced hyperuricemic rats. *A. longiloba* administration restored PO-induced liver damage. (A) Normal group, (B) hyperuricemia model group (250 mg/kg b.wt.), (C) allopurinol (5 mg/kg b.wt), (D) Fruit extract (250 mg/kg b.wt), (E) Fruit extract (500 mg/kg b.wt), (F) Petiole extract (250 mg/kg b.wt), (G) Petiole extract (500 mg/kg b.wt). All images were capture at 40x (H&E) magnification. Scale bar: 40 μm.

PO, a uricase inhibitor-drug had demonstrated an important role in pro-inflammatory responses [19] and stimulated various types of inflammatory cells, including monocytes macrophages, and neutrophils, resulting in a significant increase in the production of proinflammatory cytokines including IL-18 and IL-1β, which may induce an acute inflammation in rodents [39]. The current study revealed that the fruit and petiole extracts reduced the serum IL-18 and IL-1β levels in PO-induced HU rats. The obtained results

suggested that extracts exerted its anti-inflammation effect to prevent HU. The extracts from the petiole and fruit of *A. longiloba* used in the current study is enriched in various [30]. These results suggest that the inhibitory effect of *A. longiloba* extracts is attributable to its phytochemical compounds. Our results are in agreement with many *in vivo* studies where plant extracts rich in phytochemicals administered to the animals were effective in reducing the production of pro-inflammatory cytokines [48]. Recent work by Chen et al. [20] has reported that Curcumin from the rhizomes of *Curcuma longa* reduced the production of pro-inflammatory cytokines in the serum.

It was reported that HU might increase the burden of the kidney and liver organs and cause tissues damages [49–53]. In the present study, the histopathological examination showed that the fruit and petiole extract markedly ameliorated renal and hepatic damage in HU rats, suggesting that *A. longiloba* extracts could significantly improve kidney and liver function. In our study, the presence of inflammatory cells in the renal tissue of PO-induced rats were consistent with UA levels and XOD activity that could be due to administration of PO and HU condition. Our results are consistent with previous reports of beneficial effects of herbal extracts on kidney caused by PO [19–32]. Furthermore, the extracts protected liver tissue from damages by excessive UA levels. These results agree with the findings of Jang et al. [54], who reported the liver protective effects of *Sasa quelpaertensis* leaf extract in PO-induced hyperuricemic mice. Previous other studies reported that, PO-induced hyperuricemic rodents increased liver damages by increasing the levels of biomarkers for liver damages [44]. Therefore, we could conclude that *A. longiloba* may be used as a new potential herbal medicine to restore PO-induced liver damages and have therapeutic potential for the prevention and treatment of HU and gout.

5. Conclusion

In conclusion, the present study demonstrated that *A. longiloba* (fruit and petiole) has a UA lowering effects in hyperuricemic animals, and its mechanism of action may be related to inhibiting the activity of XOD. *A. longiloba* extracts were also found to be effective in protecting hepatic and renal tissue against PO-induced damages as the both fruit and petiole extracts showed lower concentration of hepatic cytokines; interleukin IL-1β and interleukin 18. Similarly, the extract treatments alleviated PO-induced pathological lesions. Our findings support the use of this *A. longiloba* as an alternative therapy for gout and inflammations.

Author contribution statement

Ferid Abdulhafiz: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Arifullah Mohammed: Conceived and designed the experiments; Wrote the paper; Contributed reagents, materials, analysis tools or data.

Mohd F.H Reduan: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data; Wrote the paper.

Zulhazman Hamzah: Contributed reagents, materials, analysis tools or data.

Zulhisyam Abdul Kari, Guillermo Téllez-Isaías: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

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Animal ethics approval

The animal study was approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Kota Bahru, Malaysia (Approval number: UMK/FPV/ACUC/PG/1/2020).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of abbreviations

ANOVA	analysis of variance
ARASC	Animal Research and Service Centre
CMC-Na	carboxy methyl cellulose sodium
EDTA	ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
HU	hyperuricemia
IACUC	Institutional Animal Care and Use Committee
Interleukin-1beta	IL-1β; interleukin-18- IL-18
PO	Potassium oxonate
S.E.M-	standard error of the means
UA	Uric acid
XOD	Xanthine oxidase

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