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GELAM HONEY CAN MODULATE THE ANTIOXIDANT ENZYMES ACTIVITY IN CARRAGEENAN-INDUCED PAW INFLAMMATION IN RAT

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ABSTRACT : Honey is a natural product with varied biological and medicinal properties, including antioxidant, antibacterial, antitumor and anti-inflammatory. The currentstudy aimedto examine the effect of Malaysian Gelam honey (MGH) on enzyme activity: catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) as well as malondialdehyde (MDA) plasma levels in inflammation-induced rats. Rats were pretreated with1 and 2 g/kg *p.o.* of MGH as well as with 10 mg/kg*p.o.* of Indomethacin (NSAID) for both 1 and 7 days. Subplantar injection of carrageenan 1% induced rat paw edema. Blood was taken to evaluate the MDA level and activity of antioxidant enzymes. The MGH supplementationsignificantly increased the reduction of GPx and SOD activities in inflammation-induced rats. But, the GPx and SOD activities were significantly higher with MGH at 2g/kg than 1g/kg, in both 1 and 7 days. Furthermore, MGH at 1g/kg did not affect the activity of CAT, while the CAT activity increased significantly with 2g/kg of MGH, for 1 and 7 days pretreatment. The MGH pretreatment at both 1 and 2g/kg significantly reduced the elevated of MDA levels for both 1 and 7 days. Antioxidant enzyme activities can be modulated by MGH in inflammation-induced rats, which was more notable at high concentration 2g/kg than low concentration 1g/kg.

Key words : Gelam honey, antioxidant enzyme, malondialdehyde, carrageenan, inflammation.

INTRODUCTION

Inflammation is a normal defensive immune response that occurs in reply to hurtful stimuli; including infection, damaged cells, tissue injury and toxic substances as well as against foreign pathogens (Mittal *et al*, 2014), which acts by removing the injurious stimuli then starting the process of healing (Chen *et al*, 2018). Then, there are generally two types of inflammation: acute and chronic inflammation (Silva *et al*, 2019). Acute inflammation tends to cause five specific features which are usually localized,includingredness, pain, heat and swelling, as well as the loss of function of area affected caused by the infiltration of tissues by plasma and leukocytes (Ferrero-Miliani *et al*, 2007).

Therefore, during the acute inflammatory responses, the cellular, molecular events and interactions efficiently minimize impending injury and infection.However; this alleviation process contributes to the restoration of tissue homeostasis and acute inflammation. Otherwise, uncontrolled acute inflammation may become chronic, which participatesin a variety of chronic inflammatory diseases including diabetes, cardiovascular diseases, arthritis, and cancer (Chen *et al*, 2018; Zhou *et al*, 2016).

The inflammation process is quite complex, started by several factors which comprise molecules (ranges from bacteria to chemical) and then lead to cellular shock or death. The injured tissue induced by this shock results in inflammatory mediators releasing, such as: reactive oxygen species [ROS]; like [hydrogen peroxide (H_2O_2), superoxide anions (O^{2-}) and hydroxyl radicals (OH^-)]; and cytokines (Soomro, 2019; Kumar *et al*, 2017). Thus, ROS has been involved in cell damage and apoptosis because of its oxidizing effects directly on macromolecules like proteins, lipids and DNA (Elahi *et al*, 2009).

Additionally, the polyunsaturated fatty acids react with the increased production of ROS to prompt releasing a toxic reactive aldehyde likemalondialdehyde (MDA). It is one of the lipid peroxidation process final products, that is connected with a variety of chronic health diseases (Aliahmat *et al*, 2012).

Unfortunately, the imbalance between the formation of ROS and the antioxidant mechanisms to counteract the effects of ROS or to repair the resulting damages caused oxidative stress (OS) (Pouvreau *et al*, 2018). In terms of etiology, OS is associated with several chronic

diseasessuch as: atherosclerosis,allergies, cancer, Alzheimer's and Parkinson's diseases, as well as various inflammations (Liguori *et al*, 2018). Interestingly, there is several mechanisms of antioxidant defence in the human body that regulating and blocking the production of ROS, including: (a) Endogenous antioxidants that produced by the body like antioxidant enzymes, *e.g.*, glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), *etc*. and non-enzymatic antioxidants, *e.g.*, uric acid (UA), albumin (Alb), glutathione (GSH), bilirubin (BIL), polyamines (PAs) and melatonin (MEL); (b) Exogenous antioxidants, nutrient-derived antioxidants: ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and polyphenols (Lewandowska *et al*, 2018; Zablocka and Janusz, 2008). Since OS is implicated in numerous chronic diseases, extensive studies have investigated the possible protective effect of natural products as antioxidants (Zhou *et al*, 2019).

Honey is a natural food product containing a mixture of sugars with other ingredients; such as minerals, vitamins, proteins, enzymes, flavonoids and phenolic acids as well as volatile compounds that are considered an important part of traditional medicine (Moniruzzaman *et al*, 2014). Several studies reported that honey has many therapeutic properties such as: antibacterial, antiviral, antioxidant, anticancer and anti-inflammatory (Azonwade *et al*, 2018; Zam *et al*, 2018; Hussein *et al*, 2013; Moniruzzaman *et al*, 2014). Generally, the antioxidant property of honey is probably the most important activity that may be due to both its enzymatic (peroxidase and catalase) and non-enzymatic (phenolic acids, flavonoids, ascorbic acid and products of Maillard reactions) antioxidants (Azonwade *et al*, 2018). Hussein *et al* (2011) reported the antioxidant capacity of Gelam honey who found that Gelam honey has good antioxidant properties, which attributed to its phenolic compounds (phenolic acids and flavonoids). Therefore, we aimed on this study to estimate whether Gelam honey supplementation can modulate the activity of the antioxidant enzymes during inflammation in rats.

MATERIALS AND METHODS

Gelam Honey samples

Malaysian gelam honey (MGH) is a monofloral honey produced by *Apis mellifera* from the Gelam tree (*Melaleuca cajuputi*). It was supplied by the Agriculture Department, Batu Pahat, Johor; Malaysia. The MGH was sterilized by gamma-radiation at the dose of 25 kGy in the Malaysian Nuclear Agency (SINAGAMA) by utilizing cobalt-60 source [Atomic Energy of Canada Ltd, Ontario, Canada] (Molan and Allen, 1996).

Animals

A total of eighty-four (84) Sprague-Dawley male rats (200-300 gm) were supplied by Animals Care Unit, Faculty of Medicine, The National University of Malaysia (UKM). The study was approved by the Animal Ethics Committee of the Faculty of Medicine, The National University of Malaysia {Date of approval 17th March 2010: pp/BIOK.2010/Yasmin}. Rats were kept in a well-ventilated controlled room, 12 hours light/12 hours dark cycle at temperature 22 ± 2°C, with standard rat pellet and water available *ad libitum*. Before the treatments, the rats were maintained in the room for one week (at least) to allow for acclimation within the environment of work.

Then, the inflammation was induced by the sub-plantar injection of carrageenan in the right hind paw, as described previously (Hussein *et al*, 2012). Briefly, the rats were divided into two main groups depending on the period of treatment. The first group containsthe rats who pretreated for one daywith MGH, while the second group containstherats who pretreated for seven dayswith MGH (each group has seven subgroups; n = 6 rats).The rats were orally pretreated with two doses of honey 1 and 2 g/kg(once daily), in both main groups.Additionally, the group received an equal volume of vehicle (distilled water; DW) considered as negative controlwhilethe group receivedIndomethacin (IND; 10 mg/kg), the nonsteroidal anti-inflammatory drug (NSAID), considered as a positive control (Igbe *et al*, 2010). After 1h of the last dayof MGH, DWand IND administrations, the inflammation into the right hind paw of each ratwas induced by subplantar injection of 1% freshly prepared carrageenan (0.2 ml/paw) (Kassim *et al*, 2010).

Samples preparation

After 24 hours of injection with carrageenan, the rats were anesthetized andblood was taken (around 6ml) from the orbital sinus. Then,it was maintained on ice in heparin tubes and centrifuged at 3000 rpm(15 min at 4°C). The plasma was collected and stored at -20°C for MDA determination. The erythrocytes were washed 4 times or until the supernatant becomes clear with normal saline, then aliquots and stored at -80°C until assays of the enzyme.

The SOD activity determination

The activity of SODwas carried out according to the method by Beyer and Fridovich (1987). One unit of SOD refers to the amount of enzyme required to inhibit the nitro blue tetrazolium (NBT) reduction by 50% in 1 min / ml hemolysate. The activity of SOD was expressedin units/mg of haemoglobin (U/mg Hb).

The CAT activity determination

The CAT activity was assayed according to the method described previously by Aebi (1984). CAT activity was expressed asunits/mg hemoglobin (U/mg Hb). One unit of CAT was defined as the enzyme amount needed for the H_2O_2 decomposition in phosphate buffer (pH 7.0) in onesec of reaction.

The GPx activity determination

The GPx assay was carried out using the method proposed by Paglia and Valentine (1967). One unit of GPx was defined as the amount of enzyme needed to oxidize one mmol of NADPH per g of hemoglobin (Hb) to NADP per min. Thus, the activity of GPx was expressed in milliunits/mg hemoglobin (mU/mg Hb).

Hemoglobin (Hb) determination

The levels of Hb in the blood were measured by the cyanmethemoglobin quantitative determination. Therefore, oxyhemoglobin oxidizes by ferricyanide to methemoglobin and then methemoglobin converts to cyanmethemoglobin by cyanide. The absorbance (A) at 540 nm was measured and the reaction was completed in 3 minutes.

Plasma Malondialdehyde (MDA) level

The Lipid peroxidation product MDA was measured by using high-performance liquid chromatography [HPLC] with a detector of the photo-diode array and an auto-injection valve, with some modifications (Sim *et al*, 2003). The analysis of HPLC was done by using (LC-10 AT VP) liquid chromatography system (Shidmadzu; Kyoto, Japan) and column of α-bond C18 125A (3.9x150 mm) with a particle size of 5 µm (Alltech, Deerfield, Illinois, USA). Moreover, controlled the equipment and used for processing of data was Shidmadzu Class-VP software system. The MDA level was expressed in (nmol/ ml).

Statistical analysis

The results were presented as mean ± S.E.M. (n=6), and comparison differences between groups made by using a one-way ANOVA with $p<0.05$ considered significant. The statistical analysis was carried out by the Statistical Package for Social Sciences (SPSS) version 16.0 software.

RESULTS

In the erythrocytes, SOD, CAT and GPx activities in the response to 1 & 2 g/kg of MGH, for 1 and 7 days, as illustrated in Figs. 1, 2, and 3, respectively.

The inflammation induced by carrageenan injection leads to a dramatic and significant ($p<0.05$) decrease in SOD, CAT and GPx activities in the erythrocytes compared with the control groups. However, the activities of SOD and GPx in both the 1 and 7 day time points increased significantly ($p<0.05$) with MGH administration at any dose (1 or 2 g/kg). Interestingly, pretreatment of 2 g/kg with MGH showed a greater effect on increasing the SOD and GPx activities, which was almost the same effect of the Indomethacin (NSAID drug), as shown in Figs. 1 and 3.

Moreover, 1g/kg of MGH indicated no significant ($p>0.05$) effect on CAT activity while the activity of CAT

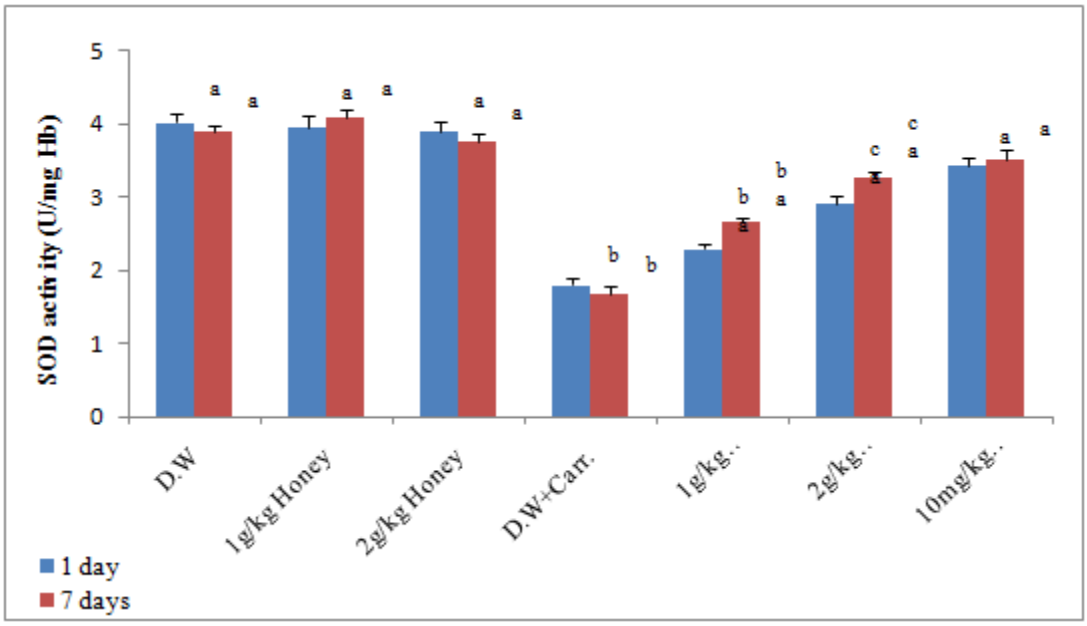

Fig. 1 : Mean values± SEM of SOD level in the erythrocytes of all study groups. [a] significant $P<0.05$ different from (D.W.+Carr.). [b]significant $P<0.05$ different from (10mg/kg IND+Carr.). [c]significant $P<0.05$ different from honey doses at the same time (1 day or 7 days). [d] significant $P<0.05$ different from time 1 and 7 days at the same honey dose.

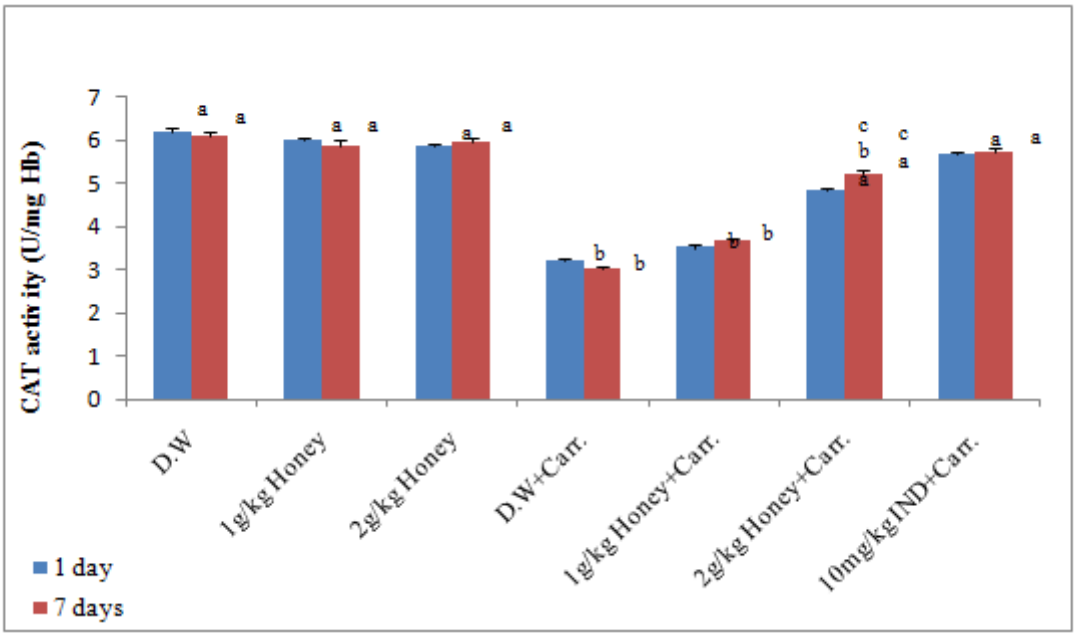

Fig. 2 : Mean values± SEM of CAT level in the erythrocytes of all study groups. [a] significant $P<0.05$ different from (D.W.+Carr.). [b]significant $P<0.05$ different from (10mg/kg IND+Carr.). [c]significant $P<0.05$ different from honey doses at the same time (1 day or 7 days). [d] significant $P<0.05$ different from time 1 and 7 days at the same honey dose.

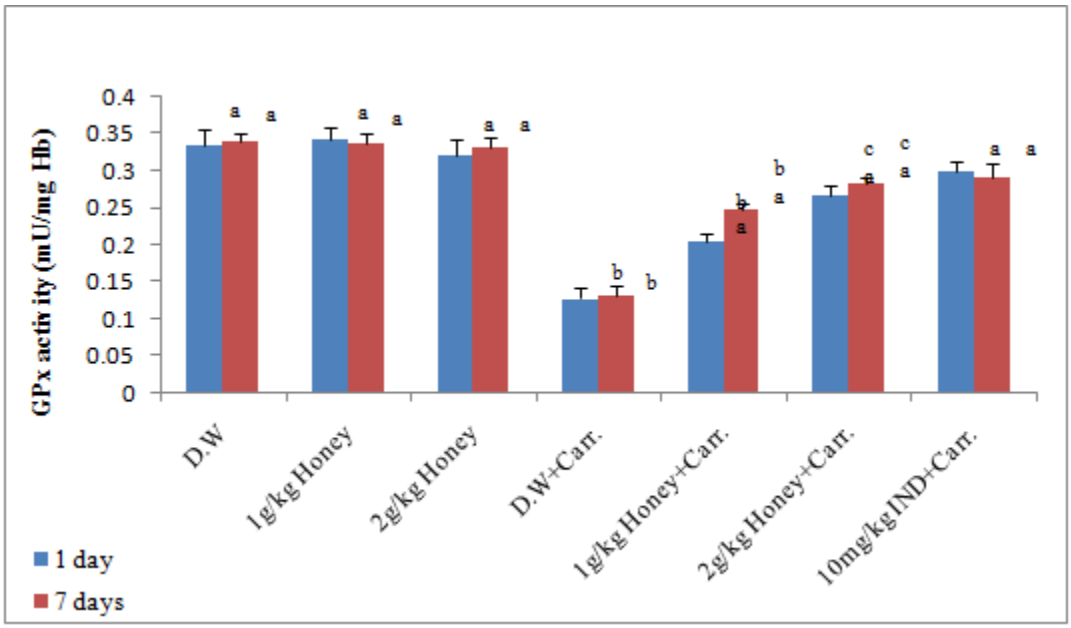

Fig. 3 : Mean values± SEM of GPx level in the erythrocytes of all study groups. [a] significant $P<0.05$ different from (D.W.+Carr.). [b]significant $P<0.05$ different from (10mg/kg IND+Carr.). [c]significant $P<0.05$ different from honey doses at the same time (1 day or 7 days). [d] significant $P<0.05$ different from time 1 and 7 days at the same honey dose.

increased significantly ($p<0.05$) with the dose of 2 g/kg, for both 1 and 7 days pretreatment (Fig. 2).

The lipid peroxidation product (MDA) levels in plasma during inflammation and pretreatment with MGH were presented in Fig. 4. We observed that there was no significant difference in MDA level for all control groups, DW and MGH at both doses 1 and 2 g/kg.

However, carrageenan injection caused a significant ($p<0.05$) elevated in plasma MDA levels in the groups of rats induced with inflammation when compared to control groups (without induced with inflammation). Remarkably, 1 and 2 g/kg pretreatment with MGH caused a significantly decreased in MDA levels while the 2 g/kg pretreatment with honey for both 1 and 7 days gave almost the same effect of Indomethacin (10mg/kg), as presented in Fig. 4.

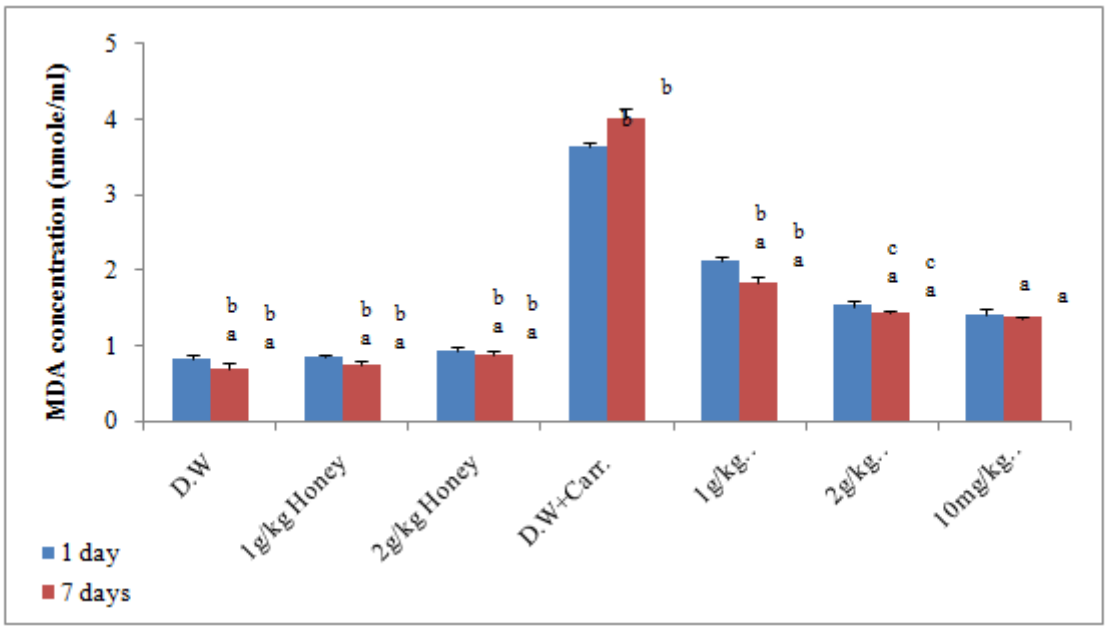

Fig. 4 : Mean values± SEM of the plasma MDA level of all study groups. [a] significant $P<0.05$ different from (D.W.+Carr.). [b] significant $P<0.05$ different from (10mg/kg IND+Carr.). [c] significant $P<0.05$ different from honey doses at the same time (1 day or 7 days). [d] significant $P<0.05$ different from time 1 and 7 days at the same honey dose.

DISCUSSION

Inflammation is one of the body's defense mechanisms against infectionor injury and also implicated in a wide range of chronic diseases when left unresolved. It can be identified by five key signs including pain, redness, swelling, heat and loss of function. Normally, the inflammation is connected with elevated levels of reactive oxygen species [ROS]; which leads tos erious damage to cells and tissues, thus ROS production plays an important role in the development of many inflammatory disorders. However, the imbalance between the ROS production and elimination led to oxidative stress development, which plays an important role in inflammatory associated diseases, like inflammatory bowel disease, atherosclerosis, neurodegenerative and cancer (Mittal *et al*, 2014; Mueller *et al*, 2010).

A dynamic equilibrium exists between the production of ROS and endogenous antioxidant defenses in normal and healthy body condition. The antioxidant systems consist of antioxidant enzymes such as SOD, CAT and GPx as well as the non-enzymatic antioxidants, including glutathione, vitamin E, vitamin C, albumin and flavonoids (Suriyaprom *et al*, 2019). Therefore, big attention has been given recently to natural products with antioxidant and anti-inflammatory activities. Since ROS play an important role in the pathogenesis of inflammatory diseases, natural antioxidants (who can scavenge ROS) are expected to combat these disorders.In the present study, the effectiveness of MGH supplementation in modulating antioxidant enzymes was investigated.Our results revealed that decreased inthe activities of SOD, CAT and GPx as well as increased MDA level with inflammation. Additionally, MGH caused a significantly increased in theGPx, SOD and CAT activities. Interestingly, MGH, particularly at 2 g/kg for 7 days, was indicated to be comparable to that effect of 10 mg/kg of Indomethacin, on increasing GPx, SOD and CAT activities.

A previous study by Yao *et al* (2011) to investigate the antioxidative effects of Gelam honey in rats (young and middle-aged) who found that the 2.5 and 5 g/kg of Gelam honey supplementation can modulate the antioxidant enzyme activities which were more eminent at higher concentration compared to lower concentration. Another study by Hasenan *et al* (2017) also reported that Gelam honey increases cardiac manganese superoxide dismutase (MnSOD) and GPx activities in young aged rats. Therefore, the capability of Gelamhoney to prevent OS in inflammation induced rats might be attributed to its antioxidants phenolic contents which can scavenge free radical activity (Chua *et al*, 2013; Hussein *et al*, 2011). Furthermore, phenolic reacts with ROS and RNS in a termination reaction, where the radical is stabilized by delocalization. Thus, breaking the cycle of the generation of new radicals as a phenolic hydroxyl group, are a good hydrogen donor (Pereira *et al*, 2009).

Moreover, in the current findings, a significant decrease was monitored in the plasma MDA level in a dose-dependent manner of Gelam honey administration. Thus, these findings show that Gelam honey can protect

inflammatory oxidation because of its antioxidant activity. Also, the reduction of MDA level is probably due to the activation of antioxidant enzymes including SOD, GPx and CAT. Similar results by Yao *et al* (2001) indicated the reduction in MDA level in both young and middle-aged rats by supplementation of Gelam honey. Otherwise, a study done by Hasenan *et al* (2017) found that increased the MDA level in the young as compared to aged rats. While supplementation of Gelam honey reduces the level of MDA in the young, but no changes were observed in the aged rats when it compared to their respective control.

According to the results obtained from the current study, might suggest Malaysian Gelam honey as a dietary antioxidant against oxidative damage.

CONCLUSION

In conclusion, this study demonstrates that experimental induction of acute inflammation in the rat by carrageenan does increase lipid peroxidation product in the plasma and does reduce the activities of antioxidant enzymes (SOD, CAT & GPx). Gelam honey supplementation, which is antioxidants rich, can alter the activities of antioxidant enzymes and reduced lipid peroxidation in a dose-dependent manner thus gives protection against oxidative stress.

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