

Full Length Research Paper

Malaysian gelam honey reduces oxidative damage and modulates antioxidant enzyme activities in young and middle aged rats

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Honey contains antioxidants such as phenolic compounds that prevent cellular oxidative damage that lead to aging, diseases and death. The aim of this study is to determine the effect of local gelam honey supplementation on oxidative status of young and middle-aged rats. Thirty-six male Sprague-Dawley rats were divided into two age groups; young (2 months) and middle aged (9 months). Each group was further divided into three groups; fed with plain water (control), supplemented with 2.5 and 5.0 g/kg of gelam honey for 30 days. DNA damage level was determined by comet assay, plasma malondialdehyde by high performance liquid chromatography and antioxidant enzymes activities (superoxide dismutase; SOD, glutathione peroxidase; GPx and catalase; CAT) were determined in the erythrocytes and liver. Results showed that supplementation of gelam honey reduced DNA damage, MDA level and GPx activity in the liver of both age groups. But erythrocytes GPx activity in young rats increased markedly with 5 g/kg of gelam honey supplementation. Liver and erythrocytes CAT activities decreased in both age groups when two different doses were used. Liver SOD activity also decreased in young rats supplemented with 5 g/kg of gelam honey. In conclusion, gelam honey reduces oxidative damage of young and middle aged rats by modulating antioxidant enzyme activities which was more prominent at higher concentration (5 g/kg body weight) compared to lower concentration (2.5 g/kg body weight).

Key words: Gelam honey, antioxidant enzymes, DNA damage, malondialdehyde.

INTRODUCTION

Oxidative stress is a condition in which the antioxidant defense system is insufficient to inactivate or neutralize oxidants such as reactive oxygen species (ROS). ROS are unstable molecules and prone to attack cellular function cells and cause oxidative damage to lipid, protein and DNA. The attack on lipids initiates a chain reaction called lipid peroxidation, which leads to generation of more ROS that can harm other cellular components. They may also cause enzyme deactivation, mutation, membrane damage, accumulation of low-density lipoproteins, disturbance in mitochondrial

function, alteration of cell function and disease leading to cellular death (Beckman and Ames, 1998). ROS are produced from various pathways of aerobic metabolism. The major source of ROS production is mitochondrial electron transport chain. The production of ROS leads to aging and diseases due to continuous damage from free radical (Sies, 1997).

Apart from that, the increased production of ROS with age also disturbs the antioxidant defense system (Sohal et al., 2002). This antioxidant defense system which function is to neutralize free radicals consist of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins A, E and C, glutathione, ubiquinone, and flavonoids. Studies have shown that honey contains all of these antioxidant compounds (Perez et al., 2006;

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Terrab et al., 2002). Honey is produced from flower nectar that has been processed by honey bees. Nuriza et al. (2005) reported that all honey consists of similar nutritional composition but different in its physiochemical content. Therefore, each honey contains different antioxidant activity due to the differences in its nectar sources.

In Malaysia, there are several types of honey such as tualang, nenas, coconut and gelam honey. Among these, tualang honey is well known in Malaysia and many studies have been done (Syukur et al., 2011; Zaid et al., 2010). Studied by Erejuwa et al. (2010) in streptozotocin-induced diabetic rats showed that tualang honey has antioxidant protective effect against oxidative stress. Krishore et al. (2011) claimed that tualang honey has higher phenolic content and greater radical scavenging activity compared with gelam honey but other study reported differently (Saba et al., 2011). Gelam honey is collected from the nectar of *Apis mellifera* which is obtained from the floral source of *Melaleuca cajuputi*. This plant can be found in the swampy coastal areas of Malaysia. A previous study claimed that gelam honey contains antibacterial activity and exhibits free radical scavenging activities due to its phenolic compounds (Aljadi and Kamaruddin, 2004). Therefore, the aim of this study is to determine the effects of gelam honey supplementation on oxidative damage and antioxidant enzymes activity of young and middle aged rats.

MATERIALS AND METHODS

Gelam honey

Malaysian gelam honey (*Melaluca* spp.) was purchased from Department of Agriculture, Batu Pahat, Johor, Malaysia. The gelam honey was packed in plastic bottles and sent to SINAGAMA, Malaysian Nuclear Agency for sterilization process using the Cobalt-60 source (Model JS10000). The irradiation process was carried out at the dose of 25 kGy. The irradiated honey was then kept in the dark at room temperature.

Animals

A total of 36 male Sprague-Dawley rats were obtained from the Animal Resources Unit, Medical Faculty, National University of Malaysia (UKM). The rats were divided into two groups; young (2 months; weighing from 200 to 250 g) and middle aged (9 months; weighing 350 to 450 g). The rats were kept in a well ventilated controlled room on a 12 h light/ 12 h dark schedule at room temperature. The rats have been kept in the room at least 1 week prior to treatment and fed with standard commercial rat pellet while drinking water was made available *ad libitum*. The experimental protocol was approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC; PP/BIOK/2010/ZAKIAH/20-JANUARY/288-MARCH-2010-APRIL-2010). Each group (young, n=18 and middle aged, n=18) was further divided into 3 groups consisting of 6 rats each; group 1 was supplemented with plain water 2.5 ml/kg body weight, group 2 with gelam honey 2.5g/kg body weight and group 3 with gelam honey 5.0 g/kg body weight. Both honey and plain water were given orally using oral gavage and the supplementation was for 30 days duration. The 2.5 g/kg body weight of gelam honey used is equivalent to 1 teaspoon of honey

as normally taken by Malaysians.

Sample preparation

After 30 days of supplementation, the rats were anaesthetized with ether and approximately 6 ml of blood were collected via orbital sinus route. Small amount of fresh whole blood were used for comet assay and the rest were centrifuged at 3000 rpm, 4°C for 10 min and the plasma obtained were divided into aliquots and stored at -80°C for MDA determination. Erythrocytes were washed three times with normal saline, separated into aliquots and stored at -80°C for enzyme assays. After the rats were killed, livers were removed and subsequently rinsed in ice cold 1.15% NaCl, (Sigma, St Louis, USA) (pH 7.2) washed and weighed. The livers were cut into small pieces and homogenates were prepared in 1.15% NaCl at 3 ml/g (w/v) using a Ultra Turrax T25 Homogenizer (IKA Labortechnik, Germany). The homogenates were centrifuged at 9000 x g for 20 min at 4°C using Sorvall RC-5B. Then the supernatant was isolated and centrifuged at 105 000 x g for 1 h using ultracentrifuge L-60 Beckman (Beckman Coulter, USA). The cytosolic supernatant was frozen at -80°C for antioxidant enzyme assays.

Detection of DNA damage by comet assay

The comet assay was performed as described by Singh et al. (1988) with slight modifications. Briefly, 5 μ L of whole blood were suspended in 0.6% low melting point agarose, pH 7.4 and rapidly pipetted onto an agarose layer and covered using a coverslip. The mixture was then allowed to solidify on an ice-cold flat tray. After removal of the coverslips, the slides were immersed into ice-cold lysing solution (2.5 M NaCl, 100 mM ethylene-diaminetetra-acetic acid, 10 mM Tris at pH 10, 1% Triton X-100, 1% sodium N-lauroyl sarcocinate, 10% dimethylsulfoxide) at 4°C for 1 h to lyse the cells and remove cellular proteins. The slides were then taken out from lysing solution and placed side-by-side in a horizontal electrophoresis chamber filled with freshly prepared cooled alkaline electrophoresis buffer (1 to 10°C) to the depth of about 0.25 cm above the agarose layer for 20 min to allow the unwinding of DNA. Electrophoresis was conducted for 20 min at 25V with the current adjusted to 300 mA by a change of buffer volume. Following electrophoresis, the slides were rinsed with neutralisation buffer 3 times and allowed to dry. Each slide was then stained with 30 μ L ethidium bromide. All the slides were examined immediately at 200x magnification using a fluorescent microscope (Olympus Corp., Shibuya-ku, Tokyo, Japan). Five hundred randomly selected non-overlapping cells on each slide were counted and grades assigned on an arbitrary scale of 0 to 4 based on perceived comet tail length migration and relative proportion of DNA in the comet tail (without tail- no damage cells, 0; cells with tiny tail, 1; cells with a dim tail, 2; cells with a clear tail, 3 and only tail, 4). For final analysis, a total damage score of each slide was calculated by using the following formula:

$$\text{Arbitrary unit} = \text{score 0} \times (N) + \text{score 1} (N) + \text{score 2} (N) + \text{score 3} (N) + \text{score 4} (N)$$

N= the number of cells assigned to each grade of damage

The sum of all grades provides a minimum possible score of 0, corresponding to 500 cells at grade 0, whereas or a maximum possible score of 2000, corresponding to 500 cells at grade 4.

Determination of SOD activity

SOD was assayed using the method developed by Beyer and

Fridovich (1987). Briefly, 1.0 ml aliquots of a mixture containing 0.1 mM phosphate buffer pH 7.8, 57 μ M nitro blue tetrazolium (Sigma, St Louis, USA), 9.9 mM L-methionine (Sigma, St Louis, USA) and 0.025% Triton-X (Sigma, St Louis, USA) were pipetted into test tubes. Then, 20 μ l of lysate or liver cytosol and 10 μ l of a solution containing 4.4 mg/100 ml riboflavin (Sigma, St Louis, USA) were added into the mixture. The tubes were illuminated for 7 min in an aluminium foil-lined box containing two 20-W Sylvania GroLux fluorescent lamps. Absorbance was then measured at a wavelength of 560 nm. A stock hemolysate was prepared by adding an equal volume of distilled water. One unit of SOD was defined as the amount of enzyme required to inhibit nitro blue tetrazolium reduction by 50% per min per ml lysate or cytosol. Enzyme activity was expressed as units per mg of Hb or protein (U/mg Hb or mg protein).

Determination of GPx activity

GPx was determined by the method developed by Paglia and Valentine (1967). The reaction mixture contained 0.05 M phosphate buffer pH 7.0, 8.4 mM NADPH (Sigma, St Louis, USA), 1.125 M sodium azide (Hopkin & William, England), 5 mM reduced glutathione (GSH), NADPH (Sigma, St Louis, USA) and 3 U/ml glutathione reductase (Sigma, St Louis, USA). The hemolysate was prepared by adding an equal volume of distilled water to the RBC pellet and allowed to stand for 1 h at 4°C. Then four parts by volume of distilled water was added. Finally, double strength Drabkin's reagent (Eagle Diagnostics, Japan) was added to yield the final hemolysate. The reaction was initiated by adding 0.1 ml of 2.2 mM H₂O₂ (Merck, Darmstadt, German). The conversion of NADPH to NADP⁺ was followed by measuring the change in O.D./min at 340 nm. One unit of GPx was defined as the amount of enzyme required to oxidize 1 μ mol NADPH/min per ml lysate or liver cytosol. Enzyme activity was expressed as milliunits per mg of Hb or protein (mU/mg Hb or mg protein).

Determination of catalase (CAT) activity

CAT was assayed by the method of Aebi (1984). The reaction mixture consisted of 50 mM phosphate buffer pH 7.0 and 30 mM hydrogen peroxide. A stock hemolysate containing 5 g Hb/100 ml was prepared by adding four parts by volume of distilled water to the sample. A 1:500 dilution of this concentrated hemolysate was prepared by adding 50 mM phosphate buffer pH 7.0 immediately before running the enzyme assay. For liver tissue preparation, 1% (v/v) triton X-100 in phosphate buffer was used in the preparation of the stock homogenate. For each one gram of organs weight, 9 ml of 1% triton X-100 was added and this stock homogenate was further diluted in phosphate buffer pH 7.0 (v: v; 1:100). Then, the reaction was started by adding 1 ml of 30 mM H₂O₂. The absorbance was measured at 240 nm. One unit of catalase enzyme was defined as the amount of enzyme which liberates half the peroxide oxygen from H₂O₂ solution in 30 s at room temperature. Enzyme activity was expressed as units per mg of Hb or protein (U/mg Hb or mg protein). Hemoglobin in the hemolysate was measured by using Eagle diagnostic kit (Japan). Protein in liver cytosol was determined using the Bradford method (1976).

Malondialdehyde level

Plasma malondialdehyde (MDA) was determined using high performance liquid chromatography (HPLC) with photo diode array detector (Shimadzu, Japan) as described by Pilz et al. (2000) with some modifications. Briefly, samples (50 μ l) were mixed with 200 μ l of 1.3 M NaOH and incubated at 60°C for 30 min. After cooling the

mixture, 100 μ l of 35% HClO₄ was added in and centrifuged at 10 000 g for 10 min. Supernatant of the samples (300 μ l) was transferred into 1.5ml of HPLC tube and 5 mM of DNPH solution (50 μ l) was added into the mixture and incubated for 30 min at room temperature. Then, samples (40 μ l) were injected into the HPLC. The amount of MDA was expressed as concentration of MDA in nmol per ml plasma.

Statistical analyses

All data were expressed as mean \pm S.D. (n=6) and differences between groups were statistically analyzed by variance (ANOVA). Differences were considered to be statistically significant if $p < 0.05$. All statistical analyses were carried out using SPSS for Windows, version 17.0.

RESULT

Determination of oxidative damage

DNA damage and plasma MDA levels were reduced with gelam honey supplementation in young and middle aged groups as presented in Figure 1. The reduction was observed in both doses of gelam honey (2.5 g/kg body weight and 5 g/kg body weight) supplementation. DNA damage reduction is not dose dependent because when the dose of gelam honey was increased to 5.0 g/kg body weight, the level of DNA damage however was almost the same as the dose of 2.5 g/kg body weight. In contrast to MDA, when the dose of gelam honey was increased, the level of MDA decreased and was significant only in the young aged group.

Antioxidant enzymes activities

Gelam honey supplementation was found to modulate antioxidant enzymes activities in erythrocytes as presented in Figure 2. Gelam honey was found to increase GPx and reduce CAT activity in both young and middle aged groups. The GPx activity increased markedly in the young group but not in middle aged group when 5.0 g/kg body weight of honey was supplemented.

In the liver, generally gelam honey supplementation reduced all the antioxidant enzyme activities (Figure 3). The reduction of GPx and CAT activities were observed in both age groups in regardless of the dose of honey supplementation but SOD activity reduced significantly in the young aged group with 5.0 g/kg body weight of gelam honey supplementation.

DISCUSSION AND CONCLUSION

DNA damage and plasma MDA levels which are the biomarkers of oxidative damage decreased significantly with gelam honey supplementation. This finding is consistent with the results obtained by Blasa et al. (2007) who claimed that honey flavanoids are able to prevent

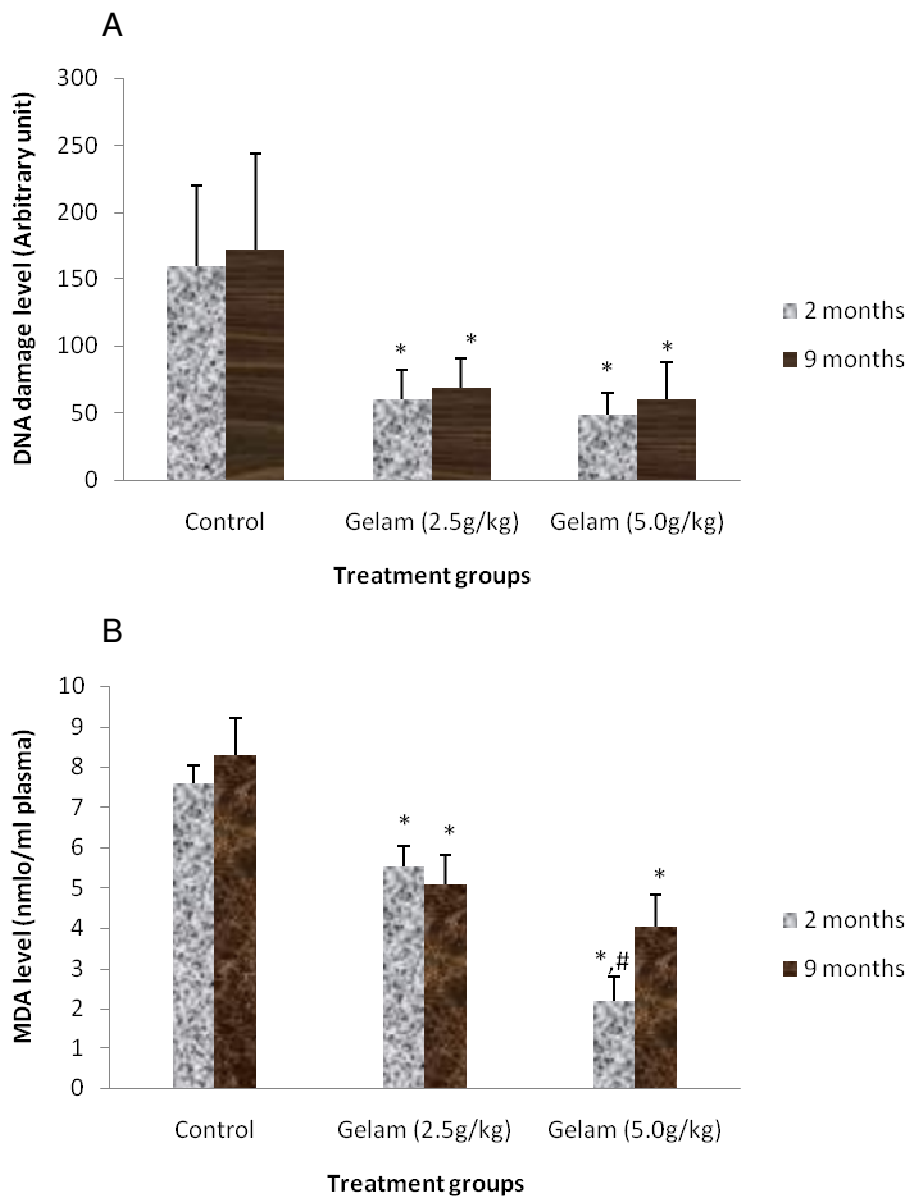


Figure 1. Effect of gelam honey supplementation on (A) DNA damage and (B) MDA levels of young (2 months) and middle aged (9 months) groups. Data are means \pm SD for 6 animals. * significant difference as compared to control group ($p < 0.05$). # significant difference as compared to gelam honey (2.5g/kg) group ($p < 0.05$).

the production of MDA in a concentration-dependent manner. The presence of phenolics in honey might have also contributed to the reduction of oxidative stress. This is so because phenolic content in honey was found to have antioxidant activity and ability to scavenge free radical activity (Kishore et al., 2011). Gelam honey was demonstrated to have higher phenolic content ($21.4 \pm 1.29 \mu\text{g/g}$ honey) as compared to coconut honey ($15.6 \pm 1.05 \mu\text{g/g}$ honey) (Aljadi and Kamaruddin, 2004). Furthermore, Saba et al. (2011) have reported that phenolic compounds in gelam honey are gallic acid, chlorogenic

acid, caffeic acid, p-coumaric acid, ferulic acid, ellagic acid, quercetin, hesperetin and chrysin. Among these, gallic acid and ferulic acid are not detected in nenas honey. These two phenolic compounds might be the unique characters of gelam honey. Gallic acid is one of phenolic compound in gelam honey has been found to be the strongest free radical scavenger. It has aromatic rings with hydroxyl group in its molecular structure which can decrease or chelate the divalent ions that act as the catalyst towards lipid peroxidation process (Perez et al., 2006). On the other hand, honey phenolics are readily

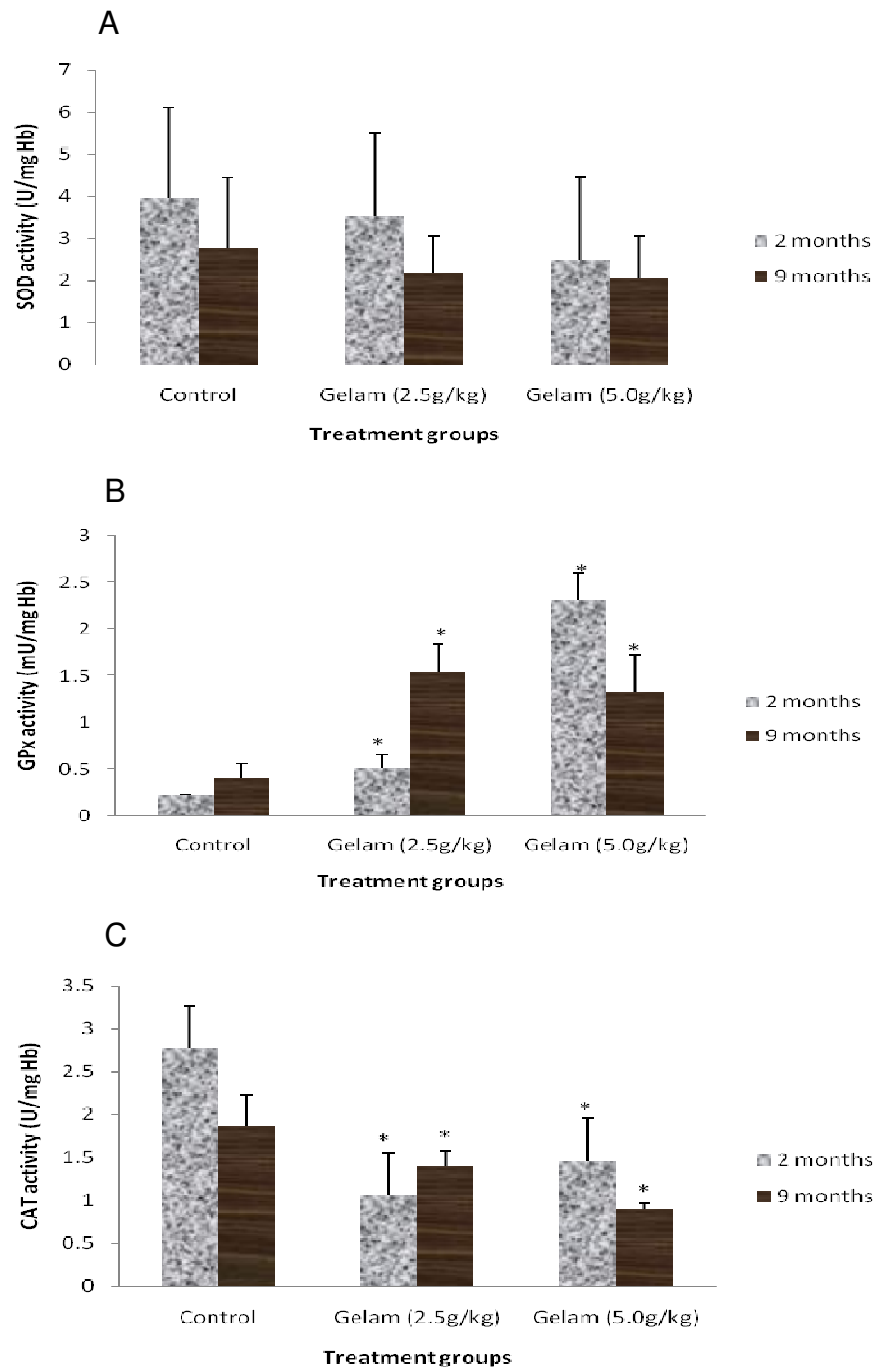


Figure 2. (A) SOD activity (B) GPx activity and (C) CAT activity in erythrocytes of young (2 months) and middle aged (9 months) rats with gelam honey supplementation. Data are means \pm SD for 6 animals. * significant difference as compared to control group ($p < 0.05$).

available and they are absorbed better through the gut barrier due to its presence in the aglyconic form produced by glycosidases present in the bee salivary glands (Gheldof et al., 2003). In contrast with other phenolics in foods or beverages such as tea which are present

in glycosidic form that requires absorption by passive diffusion. Furthermore, gelam honey which is darker in colour contains higher amount of antioxidants because studies have revealed that honey with dark colour such as buckwheat and mixed-breed honey have higher

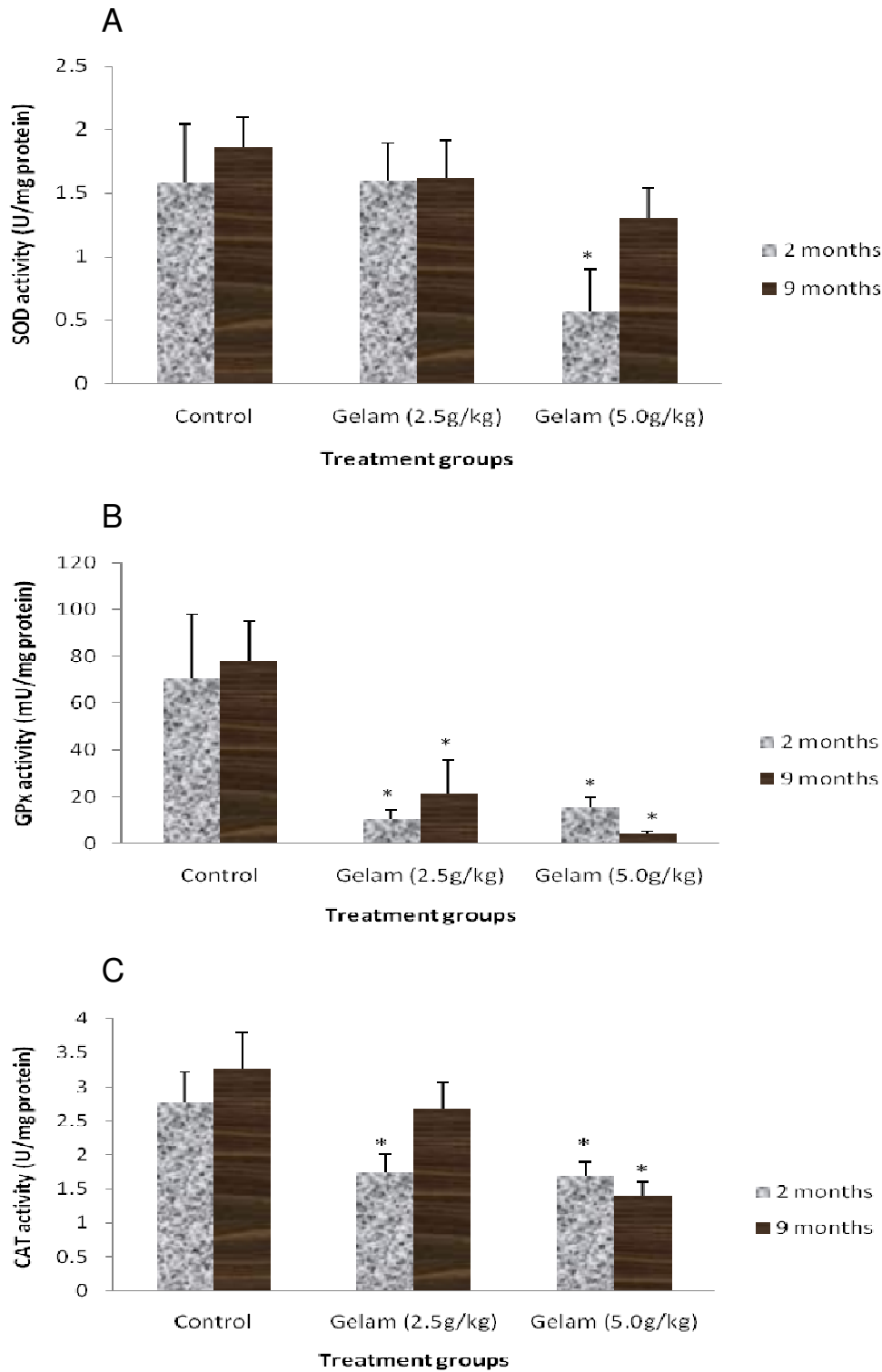


Figure 3. (A) Liver SOD activity, (B) GPx activity and (C) and CAT activity of young (2 months) and middle aged (9 months) rats with gelam honey supplementation. Data are means \pm SD for 6 animals. * significant difference as compared to control group ($p < 0.05$).

antioxidative and scavenging activities against free radicals or active oxygen species (Nagai et al., 2006;

Khalil et al., 2011). According to Aljadi and Kamaruddin (2004), an increase in the dose of honey will increase

the level of antioxidant activity. Honey supplementation modulates antioxidant enzyme activities in this study. GPx activity in the young group has increased in a dose dependent manner. This might be due to the presence of selenium in honey (Costa-Silva et al., 2011). Selenium acts as a cofactor for GPx activity. Thus supplementation of honey might increase the selenium availability in the body and thus increase GPx activity. The increased in GPx activity caused CAT activity in erythrocytes to be reduced as shown in this study. GPx and CAT act on the same substrates by eliminating hydrogen peroxide and organic peroxide (Antunes et al., 2002). In contrast to GPx activity, the activity of CAT with honey supplementation is not dose dependent.

The ability of honey to modulate antioxidant enzymes activities were supported by other studies (Erejuwa et al., 2010). Study by Kilicoglu et al. (2008) found that honey demonstrated protection in an obstructive liver jaundice due to its compounds which can acts as an anti-inflammatory agent and increasing immune defences system. Prakash et al. (2008) who used different doses of manuka honey; 5 and 10 g/kg also, found similar reduction of the colonic inflammation.

Steinberg and Witztum (2000) reported that antioxidant supplementation gave therapeutic effects towards subjects with high oxidative stress. Oxidative stress is the condition in which the cellular antioxidant defences system is insufficient to keep the levels of ROS low in the body. Many studies have reported that the oxidative stress are more common to develop in aging individual than in young people with reference to the level of MDA in this study, the response of young group after honey supplementation was better as compared to middle aged groups. This is probably due to the optimal antioxidant protection system in young group which is able to lower oxidative stress. Therefore, the supplementation is more efficient on young groups than in older age.

Natural honey contains a lot of antioxidants and its activity has been evaluated in many studies (Schramm et al., 2003). These include phenolic acids, flavonoids, ascorbic acid, carotenoid-like substances, organic acids, amino acids, proteins and enzymes. The composition and antioxidant content in honey was found mainly depends on the floral source (Gheldof et al., 2002) and also external factors such as seasonal and environment (Al-Mamary et al., 2002).

Gelam honey has been reported to have antioxidant activity, anti-inflammatory activity (Kassim et al., 2010), antibacterial activity and accelerate wound healing. These effects are due to its composition that varies in each honey and can be used as a healthy daily diet or as alternative to sugar in many products. Here, we showed that supplementation of honey give better response and protection when taken at early age as compared to latter stage of life. As a conclusion, gelam honey supplementation reduced the DNA damage and MDA level of young and middle aged rats. The action of honey to prevent oxidative damage might be due to its phenolic and

non phenolic antioxidant content or indirectly through the action of GPx activity in reducing hydrogen peroxide.

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REFERENCES

- Aebi H (1984). Catalase in vitro. In Packer L (eds) Oxygen radicals in biological systems, Academic Press, London, pp. 121-126.
- Aljadi AM, Kamaruddin MY (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chem.*, 85(4): 513-518.
- Al-Mamary M, Al-Meerri A, Al-Habori M (2002). Antioxidant activities and total phenolic of different types of honey. *Nutr. Res.*, 22(9): 1041-1047.
- Antunes F, Han D, Cadenas E (2002). Relative contributions of heart mitochondria glutathione peroxidase and catalase to H₂O₂ detoxification in *in vivo* conditions. *Free Rad. Biol. Med.*, 33(9): 1260-1267.
- Beckman KB, Ames BN (1998). The free radical theory of aging matures. *Physiol. Rev.*, 78: 547 - 581.
- Beyer WF, Fridovich I (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in condition. *Anal. Biochem.*, 161: 559-566.
- Blasa M, Candiraccia M, Accorsia A, Piacentini MP, Piatt E (2007). Honey flavonoids as protection agents against oxidative damage to human red blood cells. *Food Chem.*, 104(4): 1635 - 1640.
- Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248-254.
- Costa-Silva F, Maia M, Matos CC, Caçada E, Barros AIRNA, Nunes FM (2011). Selenium content of Portuguese unifloral honeys. *J. Food Comp. Anal.*, 24(3): 351-355
- Erejuwa OO, Sulaiman SA, Wahab MSA, Sirajudeen KNS, Salleh MSM, Gurtu S (2010). Antioxidant protective effect of glibenclamide and metformin in combination with honey in pancreas of streptozotocin-induced diabetic rats. *Int. J. Mol. Sci.*, 11(5): 2056-2066.
- Gheldof N, Wang XH, Engeseth NJ (2003). Buckwheat honey increases serum antioxidant capacity in humans. *J. Agric. Food Chem.*, 51(5): 1500-1505.
- Gheldof N, Wang XH, Engeseth NJ (2002). Identification and quantification of antioxidant components of honeys from various floral sources. *J. Agric. Food Chem.*, 50(21): 5870-5877.
- Kassim M, Achoui M, Mansor M, Yusoff KM (2010). The inhibitory effects of Gelam honey and its extracts on nitric oxide and prostaglandin E₂ in inflammatory tissues. *Fitoterapia*, 81(8): 1196-1201.
- Khalil MI, Mahaneem M, Sahil Jamalullail SM, Alam N, Sulaiman SA (2011). Evaluation of radical scavenging activity and colour intensity of nine Malaysian honeys of different origin. *J. ApiProd. ApiMed. Sci.*, 3(1): 04 - 11.
- Kilicoglu B, Gencay C, Kismet K, Serin Killcoglu S, Erguder I, Erel S, Sunay AE, Erdemli E, Durak I, Akkus MA (2008). The ultrastructural research of liver in experimental obstructive jaundice and effect of honey. *Am. J. Surg.*, 195(2): 249-256.
- Kishore RK, Halim AS, Syazana MSN, Sirajudeen KNS (2011). Tualang honey has higher phenolic content and greater radical scavenging

- activity compared with other honey sources. *Nutr. Res.*, 31(4): 322-325.
- Nagai T, Inoue R, Kanamori N, Suzuki N, Nagashima T (2006). Characterization of honey from different floral sources. Its functional properties and effects of honey species on storage of meat. *Food Chem.*, 97: 256-262.
- Nuriza NT, Arsyiah AH, Shahjahan M, Noor Izani NJ, Munavvar AS, Abdul Hye K, Mohsin SSJ (2005). Antibacterial activity of local Malaysian honey. *Mal. J. Pharma. Sci.*, 3(2): 1-10.
- Paglia DF, Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, 70 (1): 158-169.
- Perez E, Rodriguez-Malaver AJ, Vit P (2006). Antioxidant capacity of Venezuelan honey in wistar rat homogenates. *J. Med. Food*, 9(4): 510-516.
- Pilz J, Meineke I, Gleite CH (2000). Measurement of free and bound malondialdehyde in plasma by high-performance liquid chromatography as the 2,4-dinitrophenylhydrazine derivative. *J. Chrom. B: Biomedic. Sci. Appl.*, 742(2): 315-325.
- Prakash A, Medhi B, Avti PK, Saikia UN, Pandhi P, Khanduja KL (2008). Effect of different doses of Manuka honey in experimentally induced inflammatory bowel disease in rats. *Phyto. Res.*, 22(11): 1511-1519.
- Saba ZH, Kamaruddin MY, Makpol S, Yusof YAM (2011). Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of Malaysian honey. *Molecules*, 16: 6378-6395.
- Schramm DD, Karim M, Schrader HR, Roberta R, Holt RR, Cardetti M, Keen CL (2003). Honey with high levels of antioxidants can provide protection to healthy human subjects. *J. Agric. Food Chem.*, 51: 1732-1735.
- Sies H (1997). Oxidative stress: Oxidants and antioxidants. *Exp. Physiol.*, 82(2): 291-295.
- Singh NP, McCoy MT, Tice RR, Schneider EL (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.*, 175: 184-191.
- Sohal RS, Mockett RJ, Orr WC (2002). Mechanisms of aging: an appraisal of the oxidative stress hypothesis. *Free Radic. Biol. Med.*, 33(5): 575-586.
- Steinberg D, Witztum JL (2000). Is the oxidative modification hypothesis relevant to human atherosclerosis? *Circulation*, 105: 2107-2111.
- Syukur SM, Halim AS, Singh KK (2011). Evaluations of bacterial contaminated full thickness burn wound healing in Sprague Dawley rats treated with tualang honey. *Ind. J. Plast. Surg.*, 44(1): 112-117.
- Terrab A, Díeza MJ, Heredi FJ (2002). Characterisation of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chem.*, 79(3): 373-379.
- Zaid SS, Sulaiman SA, Sirajudeen KNS, Othman NH (2010). The effects of tualang honey on female reproductive organs, tibia bone and hormonal profile in ovariectomised rats-animal model for menopause. *BMC Comp. Altern. Med.*, 31(10): 82.