Food Science and Technology Research, **26** (4), 501−507, 2020

doi: 10.3136/fstr.26.501

http://www.jsfst.or.jp

Original paper

Effects of *p*-Hydroxybenzaldehyde and *p*-Hydroxyacetophenone from Non-centrifuged Cane Sugar, Kokuto, on Serum Corticosterone, and Liver Conditions in Chronically Stressed Mice Fed with a High-fat Diet

Yukiko KINJO[1], Koji WADA[2*], Moena OE[2], De-Xing HOU[3] and Makoto TAKAHASHI[2]

[1]*United Graduate School of Agricultural Science, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan*
[2]*Faculty of Agriculture, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*
[3]*Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan*

Received December 12, 2019 ; Accepted March 10, 2020

In previous studies, the non-sugar component (NSC) fraction, analyzed as 50 % methanol extract from Kokuto, showed a high *in vitro* antioxidant activity and strong anti-stress effect in acutely stressed mice. In addition, *p*-hydroxybenzaldehyde (HBA) and *p*-hydroxyacetophenone (HAP) were determined to be some of the antioxidative compounds in the NSC fraction. Therefore, the aim of the present study was to investigate whether NSC fraction, HBA and HAP could suppress stress response and the changes of hepatic lipids in chronically stressed mice fed a high fat diet. The results of the present study show that the NSC fraction has a high antioxidant activity *in vitro*, and oral administration of the fraction and HAP suppressed the secretion of stress hormone in the chronically stressed mice. We also found that the two phenolic compounds prevented hepatic lipid accumulation and peroxidation in the stressed mice.

Keywords: antioxidant activity, anti-stress effect, hepatic lipid accumulation, non-centrifuged cane sugar, *p*-hydroxyacetophenone

Introduction

Exposure to stress and fat-rich foods is common in modern lifestyles, and chronic stress is linked to an increased risk of metabolic disorders, including fatty liver disease (Ryan, 2014; Cheng *et al*., 2017). Moreover, chronic stress leads to an increase of reactive oxygen species (ROS) in hepatic lipid, which have been implicated in serious diseases (Boyd and McGuire, 1991). Prevalence of non-alcoholic fatty liver disease is associated with chronic oxidative stress, in general population is estimated to be nearly 23 % in Europe and 27 % in Asia and it is increasing remarkably over the last three decades (Younossi *et al*., 2018). Combination of stress and high-fat diet (HFD) can lead to lipid accumulation and peroxidation in liver (Kuo *et al*., 2007). The Chinese medicine formula Sinisan, called Shigyaku-san in Japan, has been shown to reduce depression-like behavior and is known to contain antioxidative compounds (Cheng *et al*., 2017; Wen *et al*., 2012). Several studies have reported that administration of the Sinisan adjusted triglyceride distribution and reduced hepatic peroxide damage in stressed non-alcoholic fatty liver disease model (Cheng *et al*., 2017). These results indicate that the utilization of dietary antioxidants with anti-stress effects can modify the chronic stress induced changes in hepatic lipids.

Non-centrifuged cane sugar, called Kokuto in Japan, has been shown to have an antioxidant effect (Payet *et al*., 2005). Kokuto may have great potential for use as an effective inhibitor of mental stress, and the Kokuto 50 % methanol fraction has been shown to have a high antioxidant activity and corticosterone suppression effect (Takahashi *et al*., 2017; Kinjo *et al*., 2019). In addition, the antioxidative compounds *p*-hydroxybenzaldehyde (HBA) and *p*-hydroxyacetophenone (HAP) were detected from the fraction, which are hypothesized

*To whom correspondence should be addressed. E-mail: kojiwada@agr.u-ryukyu.ac.jp

to have an anti-stress effect (Bountagkidou *et al*., 2010; Hacibekiroğlu and Kolak, 2011). However, so far there have been few reports concerning the anti-stress effect of Kokuto, and therefore, the aim of the present study was to investigate whether 50 % methanol non-sugar component (NSC) fraction, HBA and HAP could suppress chronic stress response in restraint stressed HFD-fed mice. We also evaluated the effect of the two phenolic compounds on hepatic lipid accumulation and peroxidation in mice.

Materials and Methods

Chemicals HBA was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). HAP, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). All other chemicals and reagents were of analytical grade.

Preparation of non-sugar component fraction Kokuto, processed from sugarcane in 2014, was obtained from Okinawa Brown Sugar Cooperative Association (Okinawa, Japan). Preparation of NSC fraction, obtained from the elusion on Diaion® HP-20 resin (Mitsubishi Chemical Corporation, Tokyo, Japan) with 50 % (v/v) aqueous methanol solution, was performed as previously described (Kinjo *et al*., 2019). As reference, the average contents of HBA and HAP in 1 g of the NSC fraction were 203 µg and 35 µg as determined by LC-MS analysis.

ABTS radical-scavenging assay and oxygen radical absorbance capacity (ORAC) assay Samples were prepared by dissolving NSC fraction, HBA and HAP in 50 % aqueous methanol. The HBA and HAP used as samples were commercially available. The ABTS radical-scavenging assay was performed as described by Miller *et al*. (1996). The ABTS radical-scavenging activity was calculated using a calibration curve of the trolox standard and expressed as µmol trolox equivalents (TE) per g of each sample. The ORAC assay was performed as described by Prior *et al*. (2003). ORAC value was determined using a calibration curve of the trolox standard and expressed as mmol TE per g of each sample.

Chronic stress test on HFD-fed mice Thirty male C57BL/6J healthy mice (7 weeks old, weighing 22.51 ± 1.05 g) were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). They were housed at 23 ± 2 ℃ with 50−60 % humidity and 12/12 h light/dark cycle in plastic cage and allowed free access to water and feed. After the adaptation period of 5 d, mice were weighted and divided into five groups based on average body weight (n=6 / group, weighing 22.82 ± 0.09 g) as follows: Blank (BLK), Control (CON), NSC, HBA, and HAP groups. All mice (except BLK group) were restrained for 60 min (10:30–11:30) a day during a period of 28 d, using a crafted restrainer trap. All mice were fed a HFD (56.7 kcal % fat, 507.6 kcal/100 g, High-Fat Diet 32, CLEA Japan, Inc., Tokyo, Japan) and weighted on days 0 and 28. Once a day, the BLK (no stress) and CON (stressed) groups were orally administrated distilled water (150 µL) and NSC, HBA, and HAP (stressed) groups were administered the respective sample dissolved in distilled water (100 mg/kg body weight) at 9:30−10:30. This dose was selected based on a previous study, which consider administration period for 28 d and non-toxic dose in mice (Kinjo *et al*., 2019). The HBA and HAP used as administration samples were commercially available. Thirty minutes after the final restraint stress, all mice were sacrificed by decapitation to minimize pain, and blood, adipose tissues (perirenal and periepididymal) and liver were collected in laboratory. The adipose tissues were weighted. This study was conducted in accordance with the guidelines of the University of the Ryukyus Policy on Animal Care and Use after approval by the Animal Care and Use Committee of the University of the Ryukyus (Okinawa, Japan). All animal care and experiments were in accordance with ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments).

Preparation of serum and liver homogenates Blood was harvested by decapitation of mice, collected in micro tubes and then allowed to stand overnight at 6 ℃. The blood samples were then centrifuged at 1 000 × g for 15 min. Obtained supernatants were centrifuged again at 3 500 × g for 5 min. The obtained serum samples were collected into tubes and stored at −30 ℃ until analysis. Excised livers were frozen instantly with liquid nitrogen after weighing and stored at −80 ℃ before homogenized. About 200 mg of the livers were homogenized by Handy micro homogenizer PHYSCOTRON NS-310E3 (Microtec Co., Ltd., Chiba, Japan) in 1 mL of phosphate buffered saline. The homogenates were centrifuged at 3 000 × g for 15 min and supernatants were collected into tubes and stored at −30 ℃ until analysis.

Measurement of corticosterone level in serum Serum corticosterone level was determined using an enzyme immunoassay kit (DetectX®, Corticosterone Enzyme Immunoassay Kit, Arbor Assays, Ann Arbor, MI, USA) according to the manufacturer's manual.

Quantitation of liver protein, triglyceride, and total cholesterol The liver protein, triglyceride (TG) and total cholesterol (TC) levels were performed according to the method described by Yamasaki *et al*. (2019). The protein concentration, as well as TG and TC levels in the supernatant of liver homogenates were determined using the Protein Quantification Kit-Rapid (Dojindo Molecular Technologies, Inc., Kumamoto, Japan), Triglyceride E test Wako and Cholesterol E test Wako (FUJIFILM Wako Pure Chemical Corporation) according to the manufacturer's instructions, respectively.

Measurement of the malondialdehyde (MDA) level in the livers MDA was determined as a thiobarbituric acid reactive substance in the liver homogenates according to the method described by Jamall and Smith (1985). Lipid peroxidation in

the livers was expressed in nmol of MDA/g protein.

Statistical analysis Experimental results are expressed as means ± SD. Data were analyzed statistically by performing one-way analysis of variance (ANOVA) and the Tukey-kramer test by using BellCurve for Excel 2012 (Social Survey Research Information Co., Ltd., Tokyo, Japan). Differences were considered statistically significant at $p < 0.05$.

Results

Antioxidant activity of NSC fraction, HBA, and HAP Table 1 shows the antioxidant activity of NSC fraction, HBA and HAP as sample characteristics. The antioxidant activity of NSC fraction, HBA and HAP were expressed as ABTS radical-scavenging activity and ORAC value. The ABTS radical-scavenging activity of NSC fraction was significantly higher ($p < 0.01$) than that of HBA and HAP. However, the ORAC level of HBA and HAP was significantly higher ($p < 0.01$) than that of NSC fraction. Therefore, NSC fraction showed antioxidant activity in ABTS radical-scavenging and ORAC assays, HAP and HBA showed high antioxidant activity in ORAC assay.

Serum corticosterone level As shown in Fig. 1, the level of serum corticosterone in the control (subjected restraint stress, CON) group significantly increased ($p < 0.01$) in comparison with the blank (not subjected restraint stress, BLK) group. Despite being exposed to chronic stress, the corticosterone levels in the non-sugar component (subjected restraint stress and treated with non-sugar component from Kokuto, NSC) and *p*-hydroxyacetophenone (subjected restraint stress and treated with *p*-hydroxyacetophenone, HAP) groups significantly ($p < 0.05$) decreased in comparison with the CON group. On the other hand, the corticosterone level in the *p*-hydroxybenzaldehyde (subjected restraint stress and treated with *p*-hydroxybenzaldehyde, HBA) group was about the same as that the level in CON group. Therefore, oral administration of NSC fraction and HAP were suppressed increase of corticosterone secretion by the chronic stress in mice.

Table 1. Antioxidant activities (ABTS radical-scavenging activity and ORAC value) of NSC fraction, HBA and HAP

	ABTS radical-scavenging activity (μmol TE/g)	ORAC value (mmol TE/g)
NSC fraction	1121.48 ± 7.19^{a}	4.77 ± 0.40^{b}
HBA	0.96 ± 0.24^{b}	27.58 ± 3.36^{a}
HAP	9.53 ± 3.00^{b}	31.32 ± 8.73^{a}

The data of ABTS radical-scavenging activity and oxygen radical absorbance capacity (ORAC) value are expressed as trolox equivalent (TE) antioxidant capacity. Value are means ± SD (n=3). For each column, different letters indicate significant differences between samples ($p < 0.05$). NSC = non-sugar component; HBA = *p*-hydroxybenzaldehyde; HAP = *p*-hydroxyacetophenone

Body weight, energy intake, adipose tissue weight and liver weight, liver protein content, liver TG and TC levels Table 2 shows body weight, energy intake, adipose tissue weight, liver weight, liver protein content, liver TG and TC levels in HFD-fed mice. At day 28, the body weight was significantly lower ($p < 0.05$) in the CON group than in the BLK group, whereas those in the NSC and HBA groups did not differ significantly from BLK group. In addition, there were no statistically significant differences between the body weights and energy intake of the groups of CON and treated mice. The results of the adipose tissue weight comparison were quite similar to the results of the body weight comparison. Also, the average liver weight in the CON group was significantly higher ($p < 0.05$) than the weight in the BLK group, whereas the liver weight in

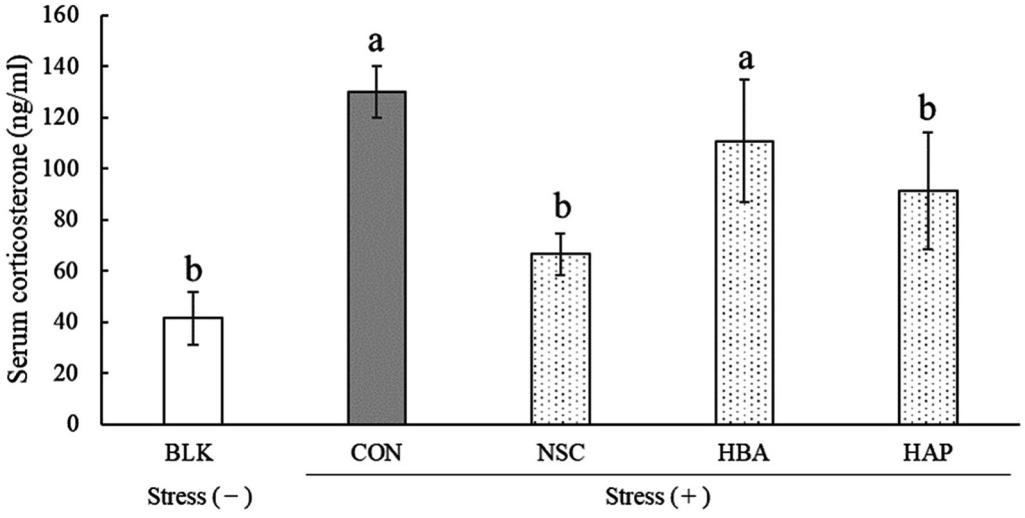

Fig. 1. Serum corticosterone levels in chronic stressed HFD-fed mice. Different letters indicate significant differences between groups ($P < 0.05$). HFD = high fat diet; BLK = blank group (no stress); CON = control group (stressed); NSC = group treatment with non-sugar component fraction (stressed); HBA = group treatment with *p*-hydroxybenzaldehyde (stressed); HAP = group treatment with *p*-hydroxyacetophenone (stressed)

Table 2. Body weight, energy intake, adipose tissue weight, liver weight, liver protein content, liver TG and TC levels in chronically stressed HFD-fed mice

	Body weight (g)		Energy intake (kcal/d)	Adipose tissue weight (g)	Liver weight (g)	Protein in liver (mg/g tissue)	TG in liver (mg/g protein)	TC in liver (mg/g protein)
	Day 0	Day 28						
BLK	22.83 ± 0.93^{a}	29.99 ± 1.75^{a}	10.94 ± 3.05^{a}	1.59 ± 0.21^{a}	1.14 ± 0.10^{b}	119.58 ± 4.68^{a}	12.23 ± 4.38^{c}	5.22 ± 0.46^{c}
CON	22.85 ± 1.18^{a}	27.01 ± 1.50^{bc}	11.42 ± 4.05^{a}	0.99 ± 0.22^{b}	1.36 ± 0.10^{a}	93.48 ± 2.40^{b}	29.48 ± 7.19^{a}	7.62 ± 0.89^{a}
NSC	22.88 ± 1.10^{a}	27.54 ± 1.27^{abc}	11.83 ± 3.94^{a}	0.88 ± 0.16^{b}	1.37 ± 0.07^{a}	108.62 ± 3.70^{a}	17.77 ± 4.05^{bc}	6.44 ± 0.15^{b}
HBA	22.88 ± 1.00^{a}	28.86 ± 1.48^{ab}	11.20 ± 3.80^{a}	1.22 ± 0.20^{b}	1.39 ± 0.15^{a}	109.81 ± 4.61^{a}	21.71 ± 3.02^{abc}	7.20 ± 0.45^{ab}
HAP	22.89 ± 0.91^{a}	26.20 ± 0.79^{c}	10.94 ± 3.45^{a}	0.89 ± 0.17^{b}	1.25 ± 0.09^{ab}	111.22 ± 11.21^{a}	26.35 ± 6.72^{ab}	6.39 ± 0.27^{b}

Values are means ± SD. For each column, different letters indicate significant differences between groups ($p < 0.05$). HFD = high fat diet; BLK = blank group (no stress); CON = control group (stressed); NSC = group treatment with non-sugar component fraction (stressed); HBA = group treatment with *p*-hydroxybenzaldehyde (stressed); HAP = group treatment with *p*-hydroxyacetophenone (stressed); TG = triglyceride; TC = total cholesterol

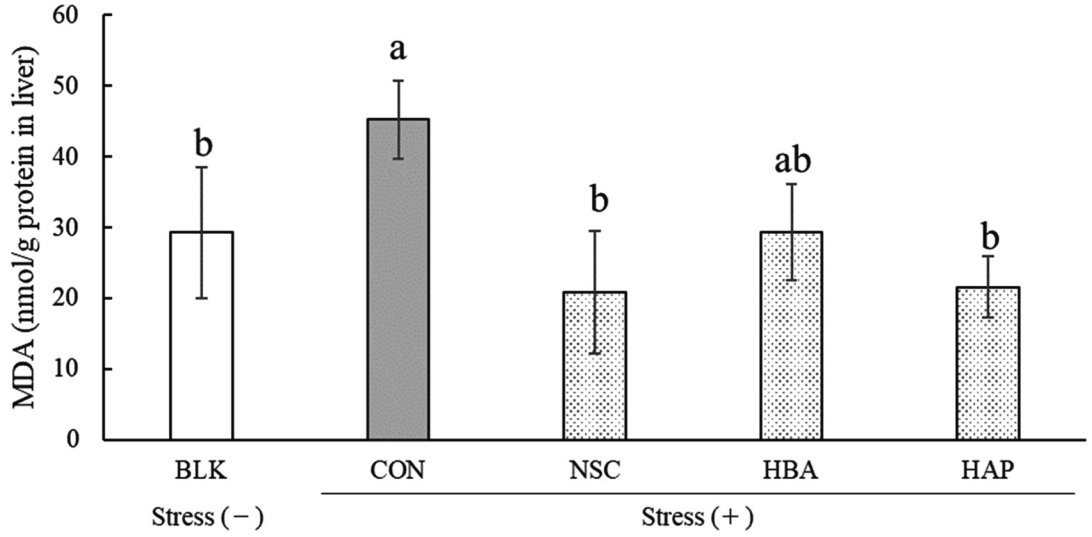

Fig. 2. Hepatic malondialdehyde (MDA) levels in chronic stressed mice fed HFD. Different letters indicate significant differences between groups ($p < 0.05$). HFD=high fat diet; BLK=blank group (no stress); CON=control group (stressed); NSC= group treatment with non-sugar component fraction (stressed); HBA= group treatment with *p*-hydroxybenzaldehyde (stressed); HAP= group treatment with *p*-hydroxyacetophenone (stressed)

the NSC, HBA and HAP groups did not differ significantly from the CON group. Although the liver protein content in the CON group significantly decreased ($p < 0.001$) in comparison to the BLK group, the contents in the NSC, HBA and HAP groups significantly increased ($p < 0.01$) in comparison with the CON group. Moreover, the levels of liver TG and TC were significantly higher ($p < 0.001$) in the CON group than in the BLK group, and the TG and TC levels in the NSC group were significantly lower ($p < 0.05$) than in the CON group. The TG level in the HBA group tended to be lower than in the CON group, and the TC level in the HAP group was significantly lower ($p < 0.05$) than in the CON group. Therefore, oral administration of NSC fraction was suppressed accumulation of TG and TC in liver induced by the chronic stress without affecting changes in adipose tissue and liver weights.

Hepatic MDA level As shown in Fig. 2, hepatic MDA level in the CON group was significantly higher ($p < 0.01$) than that in the BLK group. On the other hand, the levels in the NSC and HAP groups were significantly lower ($p < 0.05$) than that in the CON group. Oral administration of NSC fraction and HAP were suppressed increase of hepatic peroxidation in chronically stressed mice fed with a high-fat diet.

Discussion

We investigated whether NSC fraction, HBA and HAP could prevent chronic stress response, hepatic lipid accumulation and peroxidation in restraint stressed HFD-fed mice. Stress stimuli are known to lead to the secretion of glucocorticoids (cortisol in human, corticosterone in rodents) (Bale *et al*., 2000). Serum corticosterone level was significantly

higher in the CON group (stressed) than in the BLK group (no stress) (Fig. 1). Therefore, our chronic stress assay might have triggered glucocorticoid secretion by activating the hypothalamic-pituitary-adrenal (HPA) axis. Conversely, oral administration of NSC fraction and HAP suppressed the secretion of corticosterone. Watanabe *et al*. reported that antioxidative flavonoids from buckwheat sprouts exerted anti-stress property (Watanabe and Ayugase, 2008). In addition, the NSC fraction obtained from the elusion with 50 % MeOH is known to have higher antioxidant activity than that of 25 %, 75 % and 100 % MeOH on HP-20 resin, and to suppress the secretion of serum corticosterone in acute stressed mice fed with a normal diet (Kinjo *et al*., 2019). We hypothesized that the suppression of corticosterone secretion is linked to the *in vitro* antioxidant activity of the compounds. In this study, the NSC fraction also exhibited high antioxidant activity *in vitro* (Table 1). HBA and HAP also exhibited strong antioxidant activity in ORAC assay, whereas the two compounds showed weak activity in ABTS radical-scavenging assay. However, HBA and HAP has little contribution in antioxidant activity of the NSC fraction in ORAC assay because there are very few ratios of HBA and HAP (203 µg and 35 µg / g NSC fraction, respectively) for the weight of the fraction. Although the antioxidant activities of HBA and HAP present in the NSC fraction are not enough to allow us to be better understanding of the suppression of corticosterone secretion, there have been few reports about effect of HAP on stress hormone; thus, further insight into this aspect is left to future work. Administration of HBA had no effect on the secretion of serum corticosterone even though HBA has been reported to have an inhibitory effect on the HPA axis (Jung *et al*., 2006). We suggest that the results could have been affected by a multitude of factors such as administration method and stress terms.

Stress stimuli promote lipolysis and inhibit proliferation in white adipose tissue, resulting in the loss of body weight (Bowers *et al*., 2004). Here, the body weight and adipose tissues weight in chronically stressed mice significantly decreased in comparison with the BLK group (Table 2). Moreover, liver weights of the stressed mice were significantly higher in comparison with the BLK group, and the liver protein content of the CON group significantly decreased in comparison with the BLK group. Loss of liver protein could be due to injured hepatocytes resulting from oxidative stress and inflammation (Ochanda *et al*., 2016). On the other hand, the groups treated with NSC fraction, HBA and HAP showed an increase of protein content in the livers compared with the CON group. Thus, HBA and HAP may contribute to the protective effect of NSC fraction regarding liver injuries induced by chronic stress. Moreover, liver TG and TC in the CON group were significantly higher than in the BLK group. Persistent exposure to glucocorticoids induces portal circulation of many fats and promotes ectopic fat storage in the liver and vascular tissues (Dallman *et al*., 2003; He *et al*., 2017). Our data showed that serum corticosterone (Fig. 1), liver TG and TC levels (Table 2) were increased by chronic stress, indicating that hepatic lipid accumulation may be increased by persistent exposure to corticosterone. Liver TG and TC levels were significantly lower in the NSC group than in the CON group, therefore, oral administration of the NSC fraction could help prevent lipid accumulation by suppressing corticosterone secretion (Fig. 1). Moreover, oral administration of HAP had weak influence on the increase of liver TG level but prevented the increase of liver TC level (Table 2). Although the inhibitory effect towards increase of liver TG and TC is different, HAP may have anti-lipid accumulation effect. Oral administration of HBA tended to prevent the increase of liver TG level. HBA has been reported to regulate the expression of genes involved in lipid metabolism in adipocytes (Park *et al*., 2011). Therefore, HBA and HAP may contribute to the prevention effect of NSC fraction on lipid accumulation in chronically stressed mice through different mechanisms. On the other hand, consumption of fat-rich diet and exposure of chronic stress directly affect the serum lipid profile. Because the alteration of serum lipid levels affects lipid homeostasis in whole body, it would be interesting for further study to investigate the lipid levels in the serum in order to gain more insight of this phenomena.

Chronic stress is known to trigger oxidative stress, caused by increased intracellular levels of ROS (Flaherty *et al*., 2017). Oxidative damage to the liver can be confirmed by observing the level of MDA, which is a lipid peroxidation product. Hepatic MDA level in the CON group was significantly higher than in the BLK group (Fig. 2). Chronic stress in combination with fat- and sugar-enriched diet can lead to an increase of hepatic MDA levels (Fu *et al*., 2010). Oxidative stress in the liver can result in progressive cirrhosis and hepatocellular carcinoma (Kawanaka *et al*., 2004; Adams *et al*., 2005). The hepatic MDA level in the groups treated with the analyzed compounds tended to be lower than in the CON group. The *in vitro* antioxidant activity did not differ between HBA and HAP (Table 1), only the MDA level in the HAP group was significantly lower than in the CON group (Fig. 2). This difference in our results of antioxidant activity *in vitro* and *in vivo* was likely due to the bioavailability of HBA and HAP. Their metabolism in relation to the active or inactive form and the co-ingestion of other nutrients affect their bioavailability (Martins *et al*., 2016). The inhibitory effect of HAP on the increase of hepatic MDA is caused by increasing the activity of antioxidant enzymes (Chang *et al*., 2017). Hence, administration of NSC fraction prevented hepatic lipid peroxidation induced by chronic stress, and this effect could be contributed to the activation of antioxidant enzymes *in vivo* by administration of HAP.

In conclusion, we investigated the effect of NSC fraction, HBA and HAP on chronic stress responses, and found that oral administration of NSC fraction suppressed the increase of corticosterone secretion in chronically stressed HFD-fed mice.

Oral administration of the NSC fraction prevented not only hepatic lipid accumulation but also hepatic lipid peroxidation. These mechanisms were possibly mediated by the antioxidant activity *in vivo* of HAP. Evaluation of other compounds besides HAP which could influence the suppression effects of NSC fraction are now in progress because other antioxidant phenol compounds detected from the NSC fraction in previous study (Kinjo *et al*., 2019). This is the first study showing that HAP may contribute to the anti-stress effects of Kokuto, and the present findings support the utilization of Kokuto as a functional food for the prevention of stress and lipid metabolic disease.

Acknowledgments This work was partially supported by a Grant-in-Aid for Scientific Research (17K00819) from Japan Society for the promotion of Science.

References

Adams, L.A., Lymp, J.F., Sauver, J.S., Sanderson, S.O., Lindor, K.D., Feldstein, A., and Angulo, P. (2005). The natural history of nonalcoholic fatty liver disease: A population-based cohort study. *Gastroenterol*ogy, **129**, 113–121.

Bale, T.L., Contarino, A., Smith, G.W., Chan, R., Gold, L.H., Sawchenko, P.E, Koob, G.F., Vale, W.W., and Lee, K.F. (2000). Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behavior and are hypersensitive to stress. *Nat. Genet.*, **24**, 410–414.

Bountagkidou, O.G., Ordoudi, S.A., and Tsimidou, M.Z. (2010). Structure–antioxidant activity relationship study of natural hydroxybenzaldehydes using *in vitro* assays. *Food Res. Int.*, **43**, 2014–2019.

Bowers, R.R., Festuccia, W.T.L., Song, C.K., Shi, H., Migliorini, R.H., and Bartness, T.J. (2004). Sympathetic innervation of white adipose tissue and its regulation of fat cell number. *Am. J. Physiol.: Regul., Integr. Comp. Physiol.*, **286**, 1167–1175.

Boyd, N.F. and McGuire, V. (1991). The possible role of lipid peroxidation in breast cancer risk. *Free Radical Biol. Med.*, **10**, 185–190.

Chang, C.W., Chen, Y.C., Lin, Y.C., and Peng, W.H. (2017). *p*-Hydroxyacetophenone suppresses nuclear factor-κB-related inflammation in nociceptive and inflammatory animal models. *J. Nat. Med.*, **71**, 422–432.

Cheng, F., Ma, C., Wang, X., Zhai, C., Wang, G., Xu, X., Mu, J., Li, C., Wang, Z., Zhang, X., Yue, W., Du, X., Lian, Y., Zhu, W., Yin, X., Wei, Z., Song, W., and Wang, Q. (2017). Effect of traditional Chinese medicine formula Sinisan on chronic restraint stress-induced nonalcoholic fatty liver disease: a rat study. *BMC Complementary Altern. Med.*, **17**, 203–212.

Dallman, M.F., Pecoraro, N., Akana, S.F., Fleur, S.E., Gomez, F., Houshyar, H., Bell, M.E., Bhatnagar, S., Laugero, K.D., and Manalo, S. (2003). Chronic stress and obesity: A new view of "comfort food". *PNAS*, **100**, 11696–11701.

Flaherty, R.L., Owen, M., Fagan-Murphy, A., Intabli, H., Healy, D., Patel, A., Allen, M.C., Patel, B.A., and Flint, M.S. (2017). Glucocorticoids induce production of reactive oxygen species/reactive nitrogen species and DNA damage through an iNOS mediated pathway in breast cancer. *Breast Cancer Res.*, **19**, 35.

Fu, J.H., Sun, H.S., Wang, Y., Zheng, W.Q., Shi, Z.Y., and Wang, Q.J. (2010). The effects of a fat- and sugar-enriched diet and chronic stress on nonalcoholic fatty liver disease in male Wistar rats. *Dig. Dis. Sci.*, **55**, 2227–2236.

Hacibekiroğlu, I. and Kolak, U. (2011). Antioxidant and anticholinesterase constituents from the petroleum ether and chloroform extracts of *Iris suaveolens*. *Phytother. Res.*, **25**, 522–529.

He, Z., Lv, F., Ding, Y., Huang, H., Liu, L., Zhu, C., Lei, Y., Zhang, L., Si, C., and Wang, H. (2017). High-fat diet and chronic stress aggravate adrenal function abnormality induced by prenatal caffeine exposure in male offspring rats. *Sci. Rep.*, **7**, 14825–14833.

Jamall, I.S. and Smith, J.C. (1985). Effects of cadmium on glutathione peroxidase, superoxide dismutase, and lipid peroxidation in the rat heart: a possible mechanism of cadmium cardiotoxicity. *Toxicol. Appl. Pharmacol.*, **80**, 33–42.

Jung, J.W., Yoon, B.H., Oh, H.R., Ahn, J.H., Kim, S.Y., Park, S.Y., and Ryu, J.H. (2006). Anxiolytic-like effects of *Gastrodia elata* and its phenolic constituents in mice. *Biol. Pharm. Bull.*, **29**, 261–265.

Kawanaka, M., Mahmood, S., Niiyama, G., Izumi, A., Kamei, A., Ikeda, H., Suehiro, M., Togawa, K., Sasagawa, T., Okita, M., Nakamura, H., Yodoi, J., and Yamada, G. (2004). Control of oxidative stress and reduction in biochemical markers by Vitamin E treatment in patients with nonalcoholic steatohepatitis: a pilot study. *Hepatol. Res.*, **29**, 39–41.

Kinjo, Y., Takahashi, M., Hirose, N., Mizu, M., Hou, D.X., and Wada, K. (2019). Anti-stress and antioxidant effects of non-centrifuged cane sugar, Kokuto, in restraint stressed mice. *J. Oleo Sci.*, **68**, 183–191.

Kuo, L.E., Kitlinska, J.B., Tilan, J.U., Li, L., Baker, S.B., Johnson, M.D., Lee, E.W., Burnett, M.S., Fricke, S.T., Kvetnansky, R., Herzog, H., and Zukowska, Z. (2007). Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nat. Med.*, **13**, 803–811.

Martins, N., Barros, L., and Ferreira, I.C.F.R. (2016). *In vivo* antioxidant activity of phenolic compounds: Facts and gaps. *Trends Food Sci. Technol.*, **48**, 1–12.

Miller, N.J., Sampson, J., Candeias, L.P., Bramley, P.M., and Rice-Evans, C.A. (1996). Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.*, **384**, 240–242.

Ochanda, S.O., Rashid, K., Wanyoko, J.K., Ngotho, M., Faraj, A.K., Onyango, C.A., Wachira, F.N., and Maranga, D.N. (2016). Fortification of alcoholic beverages (12% v/v) with tea (*Camellia sinensis*) reduces harmful effects of alcohol ingestion and metabolism in mouse model. *BMJ Open Gastro.*, **3**, 1–9.

Park, S., Kim, D.S., and Kang, S. (2011). *Gastrodia elata* Blume water extracts improve insulin resistance by decreasing body fat in diet-induced obese rats: vanillin and 4-hydroxybenzaldehyde are the bioactive candidates. *Eur. J. Nutr.*, **50**, 107–118.

Payet, B., Sing, A.S.C., and Smadja, J. (2005). Assessment of

antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: determination of their polyphenolic and volatile constituents. *J. Agric. Food Chem.*, **53**, 10074–10079.

Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., and Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. *J. Agric. Food Chem.*, **51**, 3273–3279.

Ryan, K.K. (2014). Stress and metabolic disease. In "Sociality, hierarchy, health comparative biodemography: A collection of papers", ed. by M. Weinstein, and M.A. Lane. National Academies Press, Washington, D.C., pp. 247–268.

Takahashi, M., Kinjo, Y., Uechi, S., Hirose, N., Mizu, M., Hou, D.X., and Wada, K. (2017). Effects of oral intake of noncentrifugal cane brown sugar, Kokuto, on mental stress in humans. *Food preserv. Sci.*, **43**, 123–132.

Watanabe, M. and Ayugase, J. (2008). Anti-stress effects of flavonoids from buckwheat sprouts in mice subjected to restraint stress. *Food Sci. Technol. Res*., **14**, 253–260.

Wen, J., Qiao, Y., Yang, J., Liu, X., Song, Y., Liu, Z., and Li, F. (2012). UPLC–MS/MS determination of paeoniflorin, naringin, naringenin and glycyrrhetinic acid in rat plasma and its application to a pharmacokinetic study after oral administration of Si-Ni-San decoction. *J. Pharm. Biomed. Anal.*, **66**, 271–277.

Yamasaki, K., Sugamoto, K., Arakawa, T., Nishiyama, K., and Yamasaki, M. (2019). Chronic intake of high-dose of blueberry leaf extract does not augment the harmful effects of ethanol in rats. *PeerJ*, **7**, e6989.

Younossi, Z., Anstee, Q.M., Marietti, M., Hardy, T., Henry, L., Eslam, M., George, J., and Bugianesi, E. (2018). Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.*, **15**, 11–20.