Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Pharmacology and Toxicology

ISSN 1816-496X
DOI: 10.3923/jpt.2016.1.10

Research Article
Hepatoprotective Effects of Bitter Melon and Blueberry Leaf Teas on Endogenous Hepatic Antioxidant Enzymes

[1,2]S. Willis, [1,2]J. Boateng, [1]K. Busambwa, [2]L. Shackelford and [2]M. Verghese

[1]Food and Nutritional Toxicology and Safety Laboratory, Department of Food and Animal Sciences, Alabama A and M University, 35762 Normal AL, USA
[2]Nutritional Biochemistry and Carcinogenesis Laboratory, Department of Food and Animal Sciences, Alabama A and M University, P.O. Box 1628, 35762 Normal Al, USA

Abstract

Background and Objective: Demand for plant preparations is on the rise urging the need to investigate their potential for toxicity. The aim of this study was to evaluate the effect of oral supplementation of bitter melon (BMLT) and blueberry leaf teas (BLUT) on redox and drug metabolizing enzymes: Glutathione (GSH) levels, Glutathione-S-Transferase (GST), superoxide dismutase (SOD), catalase (CAT) activities were determined. **Methodology:** Rats (n = 5) were administered once daily by gavages 0, 1000, 2000 and 4000 mg kg^{-1} b.wt., of BMLT, BLUT for 28 days. Day 29, rats were killed by CO_2 asphyxiation and tissue and blood samples were collected for analysis. **Results:** Results also showed GST, CAT and SOD (μmol mg^{-1} protein) activities in experimental groups ranged from 0.89-2.14, 0.93-1.38 and 0.35-0.55, respectively. There were no significant differences in GSH (μmol/mg protein) levels between the control and treatment groups. **Conclusion:** Results indicated BMLT and BLUT prevented oxidative stress by improving the activity of antioxidant enzymes. Results also showed no apparent toxicity in rats administered LHT.

Key words: Antioxidants, bitter melon leaf, blueberry leaf, herbal leaf teas, endogenous enzymes

Received: February 02, 2016 Accepted: April 30, 2016 Published: June 15, 2016

Citation: S. Willis, J. Boateng, K. Busambwa, L. Shackelford and M. Verghese, 2016. Hepatoprotective effects of bitter melon and blueberry leaf teas on endogenous hepatic antioxidant enzymes. J. Pharmacol. Toxicol., 11: 1-10.

Corresponding Author: Judith Boateng, Department of Food and Animal Sciences, Alabama A and M University, P.O. Box 1628, 35762 Normal AL, USA Tel: 256-372-8794 Fax: 256-372-5432

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

With a growing list of side effects ascribed to prescription drugs, the use of herbal medicine and supplements is steadily increasing. Approximately, one third to half of medicines available on the market is derived from plant or natural sources (Dasgupta, 2010). According to Schoepfer *et al*. (2007), over 60% of patients in the US reported the use of dietary supplements and approximately 80% of the world's population depends on traditional herbal medicines for their primary health care (WHO., 2008). In the US alone, the market for herbal medicines increased from $200 million to $3.3 billion from 1988 to 1997 (Mahady, 2001) and in a study conducted by Barnes (2002), 18.9% of participants used natural products.

Blueberry and bitter melon leaf teas are gaining popularity as Leafy Herbal Teas (LHT). According to Zhao *et al*. (2013), herbal tea is a commonly consumed beverage brewed from the leaves, flowers, seeds, fruits, stems or roots of plant species other than *Camellia sinensis* L. The reasons for LHT use may vary with the different cultures of each individual society. However, it can basically be considered as being either for the maintenance of health, treatment or prevention of minor ailments (Deng, 2002; Barnes, 2003; Critchley *et al*., 2005)and also for chronic illnesses (Sydara *et al*., 2005; Chang *et al*., 2007; Mehta *et al*., 2007). The LHT are purported to have several therapeutic properties, which include, blood purifying properties, purgative, laxative and stimulant,as well as an aphrodisiac.

Accounting for these potential benefits is a diverse array of compounds with numerous biological properties, such as antioxidant, anti-inflammatory, anti-carcinogenic, anti-atherogenic, anti-aging, cardioprotective, chemopreventive, hepatoprotective and neuroprotective activities (Zhao *et al*., 2013). Study suggests these health benefits are attributed in large part to the presence of phytochemicals (Kubola and Siriamornpun, 2008). Even so, these compounds when taken in large amounts may have adverse effects on health (Bun *et al*., 2006; Cupp, 1999; Ridker, 1987). Furthermore, with their wide usage and easy availability, the safety and toxicity of LHTs has become an issue of concern.

Reports of adverse reactions (i.e., allergic reactions) and potential interactions with pharmaceuticals or prescriptions drugs have resulted in both acute and chronic intoxications. While the public and some health care professionals believe that LHT are rather safe because they are "Natural", there are little data to support this assumption (Issa *et al*., 2006). Therefore, more study needs to focus on the possible toxic effects of these substances in the interest of public health. While, the toxicity of many herbal remedies remains unknown, understanding them beyond their benefits is also needed, as well as their possible adverse effects. From a scientific view point, this is crucial when considering the fact that this niche market is left largely unregulated (Dipaola *et al*., 1998; Halsted, 2003).

Despite the documented health benefits of blueberries and bitter melon fruits, little attempt if any has been made to investigate the potential health properties ascribed to their herbal leaf infusions; further, very little information exists with regard to their potential harmful effects, especially when consumed in large quantities. The current study was undertaken due to limited information on the safety and effect on endogenous antioxidant enzymes of LHT, particularly blueberry and bitter melon leaf teas. Thus, the objectives were to determine the hepatoprotective effect of oral supplementation of blueberry leaf and bitter melon leaf teas on the modifications of endogenous antioxidant liver enzymes in a rodent model.

MATERIALS AND METHODS

Acquisition of teas and other materials: Blueberry leaf teas were purchased from Monterey Bay Spice Company (Watsonville, CA). Bitter melon leaf tea was prepared from bitter melon leaves obtained from the Winfred Thomas Agricultural Research Station (WTARS), Alabama Agricultural and Mechanical Agricultural (AAMU) Station in Hazel Green, AL. Dietary ingredients for preparing AIN 93G were obtained from ICN (Costa Mesa, CA). The diets were mixed every week and stored at 4°C until fed to experimental animals. All chemicals used were of analytical grade and were obtained from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Suwanee, GA).

Preparation of tea decoctions: To prepare aqueous extracts of herbal leaf teas, 50 g of whole leaves were boiled with 250 mL of distilled water under reflux for 30 min. The tea leaves were extracted a second time with 250 mL of distilled water. The decoctions were combined, cooled to room temperature and then filtered. The decoctions were stored at -20°C until use (tea decoctions were also used for TPC, TFC and FRAP analysis). Rats were administered doses of 1000, 2000 and 4000 mg kg^{-1} b.wt., of herbal tea extracts. Extracts were diluted in distilled water and administered by gavages (using a 22G intubation needle) at a rate of 0.1 mL/10 g body weight of herbal tea once daily for 28 days. In order to provide

the correct mg/kg/daily dose, the doses were modified continually according to recently recorded weight.

Animal housing and diets: An *in vivo* model of sub-acute toxicity study was utilized for the assessment of sub-acute toxicity. A total of 35 fisher 344 male rats (Harlan, IN) at 4 weeks of age were housed in an environmentally controlled animal care facility for the duration of the study. At 7 weeks, rodents were randomly assigned to groups each consisting of 5 rats. All deviations in general behavior associated with the administration of test compounds were observed for the onset of any immediate toxic signs and also during the observation period to record any delayed acute effects. Treatments were given daily for 28 days consecutively, LHT were administered between 10 AM and 12 noon each day. At the end of the testing period (day 29), all rats were fasted for 16-18 h and a single collection of blood was taken followed by immediate euthanasia by carbon dioxide asphyxiation. Vital organs of each rat were excised, blotted and weighed and the organ/body weight ratios calculated. Rodents had *ad libidum* access to food (AIN-93G) (Reeves *et al*., 1993a, b) and water. The animals were handled and the study was conducted in accordance with the AAMU guidelines for the protection and care of animals. Institute of Animal Care and Use Committee (IACUC) committee at AAMU approved protocol for the study.

Clinical parameters: Attention was paid daily to sensitivity of parameters such as survival, dramatic weight changes and changes in organ/tissues and feed consumption. Weekly feed and water consumption were recorded over the study duration. Signs including changes in skin, fur, eyes and mucous membranes, presence of secretions and excretions, response to handling and presence of clonic or tonic movements were noted.

Preparation of liver for analytical measurements: One gram of liver was homogenized in 10 mL of tris hydrochloric acid mixture. Following homogenization, liver homogenate was centrifuged for 20 min at 5,000xg. Supernatant was decanted into test tubes. Following decantation, the precipitant was washed with an additional volume (10 mL) of tris hydrochloric acid and centrifuged for 20 min at 10,000xg.The supernatant was collected and used for analysis.

Redox enzymes and phase II metabolizing enzyme determinations: Catalase (CAT) activity was measured by the reaction of formaldehyde produced from methanol with purpald to produce a chromophore according to the method of Johansson and Borg (1988). Quantitation was carried out by measuring the absorbance at 540 nm and comparing the results with those obtained with formaldehyde calibrators and expressed as μmol mg^{-1} protein. Glutathione (GSH) from cell (μmol mg^{-1} protein) was determined according to Griffith (1980). For superoxide dismutase (SOD) determination the method outlined by Fernandez-Urrusuno *et al*. (1997) was employed. Xanthine oxidase was utilized to generate a superoxide flux and samples will be measured at 440-505 nm after addition of xanthine oxidase. Data was expressed as units of SOD activity per mg protein. For glutathione-S-transferase (GST) activities, the method of Habig *et al*. (1974) was modified for the microplate reader. The 1-chloro-2, 4-dinitrobenzene was used as the substrate and GST calculated as μmol mg^{-1} protein. Samples were analyzed in triplicates.

Determination of total phenolics and flavonoids contents in herbal tea extracts: Total phenolic content in blueberry leaf and bitter melon leaf tea (prepared as previously described) was determined based on the Folin-Ciocalteau (FC) method (Singleton *et al*., 1999) and modified for the microplate (Gajula *et al*., 2009). Briefly, in 96-well plate, 12.5 μL of sample was combined with 50 μL of distilled water and 12.5 μL of FC reagent. Samples were incubated for 5 min at room temperature and 125 μL of 7% Na_2CO_3 was added. Reaction mixture was incubated for 90 min at ambient temperature; after which the absorbance was read at 750 nm in a micro plate reader (Synergy HT, BioTek instruments, USA). Results were compared to gallic acid standard and expressed as milligram gallic acid equivalents/100 mL of herbal leaf tea.

Total flavonoids in tea extracts were determined based on aluminum chloride colorimetric method described by Zhishen *et al*. (1999) with modifications, Gajula *et al*. (2009). Briefly, in a 96-well plate 25 μL of sample was combined with 125 μL of distilled water and 7.5 μL of $NaNo_2$. After an incubation period of 5 min at room temperature, 15 μL of 10% $AlCl_3$ was added. The reaction mixture was incubated for additional 5 min at room temperature. After the incubation period, 50 μL of 1 M NaOH and the mixture was immediately diluted by the addition of 27.5 μL. Absorbance of the mixture was measured at 510 nm against a blank and results were compared to a catechin standard and expressed as milligram of catechin equivalents/100 mL of herbal leaf tea.

Determination of Ferric Reducing Antioxidant Power (FRAP) of herbal tea extracts: The FRAP assay for blueberry leaf and bitter melon leaf teas was determined by the method described by Benzie and Strain (1999) and modified for the

micro plate. Briefly, FRAP solutions A (acetic acid buffer), B (TPZ) and C (ferric chloride solution) were mixed at a 10:1:1 ratio to form the FRAP reagent. In 96-well plate, 10 µL of sample were combined with 300 µL of H_2O and 30 µL of FRAP reagent. The mixture was subsequently incubated for 10 min at 37°C. Following incubation, the samples were analyzed at an absorbance of 593 nm. The change in absorbance was compared to a to a standard ferrous sulphate ($FeSO_4.7H_2O$) (0.1-1.0 mM). The samples were analyzed in triplicates and the concentration of Fe^{3+} was expressed as micromole of Fe^{2+}/100 mL of herbal leaf tea.

Statistical analysis: Experiments were analyzed in triplicates. Data were analyzed using the SAS statistical (SAS 9.2) program (SAS., 2013). Results were performed by ANOVA and values are given as Means±SEM. Means were separated using Tukey's studentized range test. Differences between treatment groups were tested by student's t-test and paired t-test. Unless otherwise indicated levels of significance were considered significant at $p<0.05$.

RESULTS

Clinical observations: During the treatment period, no adverse signs of toxicity were observed. All animals appeared healthy, as there was no hair loss or changes in skin, fur, eyes and mucous membranes. There were also no occurrence of secretions and excretions. Animals were periodically (daily) observed for autonomic activity and gait and no changes were observed. Animals responded calmly to handling and no clonic or tonic movements and behavior were noted.

Effect of Herbal Leafy Teas (LHT) on weight gain, feed in take and relative organ weights: Body weight gain as a function of dose is shown in Table 1. All the groups showed weight gains compared with their initial weight. Weight gains were significantly ($p\leq0.05$) lower inthe BLUT treated group compared to BMLT group and the CON. Among the treated groups, rats administered BMLT had the highest ($p\leq0.05$) weight gains. In the BMLT group weight gain were decreased by 24-40% compared to CON and for rats given BLUT weight gain were decreased by 64-72%. In their study, Raza *et al*. (2000), explained that changes in body weight could indicate adverse effects when the animal shows a loss greater than 10% of initial weight. None of the groups in this study showed weight loss greater that 10%. In fact, all rats in experimental groups gained weight (Table 1). Corrected daily feed intakes were also recorded and are shown in Table 1. Feed intake in CON was significantly ($p\leq0.05$) lower (15.52±0.11) compared to the treated groups. There was however, no significant difference in feed intake among the rats administered leafy herbal teas.

The most frequent measure used to interpret the effects of dietary components on organ weights in toxicological experiments, is the ratio of the organ weight to the animal's body weight, i.e., relative organ weights. Herbal leafy tea (BLUT and BMLT) administration did not have any adverse effect on the final weights (g) and the Relative Weights (RWs) of organs (g) in the rats (Table 2). The RWs of organs of the treatment groups were similar ($p\leq0.05$) to the control, except for RW of liver, which was decreased ($p\leq0.05$) for the BMLT-4 group. The RW for the lung was increased ($p\leq0.05$) in the group administered BMLT-1 and decreased in the group administered BLUT-2 when compared to the control.

Effect of herbal leafy teas on the modulation of phase II detoxification enzyme (glutathione-S-transferase (GST) and redox enzymes such as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD)

Glutathione-S-transferase (GST) activity: Glutathione-S-transferase (GST) activity can be used as an indicator of toxicity of dietary compounds. In this study, the effects of the administration of different concentrations of leafy herbal teas on GST activity are shown in Fig. 1a. Results showed that there were no significant differences in GST (µmol mg^{-1} protein) activity between the treated groups and CON, with the

Table 1: Daily feed intakes and weight gain in rats administered herbal leafy teas (blueberry leaf (BLUT) and bitter melon leaf (BMLT))

Treatments	Feed intake (g day^{-1})	Initial weight (g)	Final weight (g/28 days)	Weight gain (g)	Weight gain (%)
Control	15.52±0.11^{b}	286.50±0.64^{a}	305.50±3.68^{a}	19.00±2.34^{a}	6.62
BLUT-1	19.59±0.31^{a}	286.75±5.66^{a}	293.50±5.5^{ab}	6.75±9.05^{d}	2.35
BLUT-2	20.54±2.50^{a}	284.50±9.31^{a}	289.70±4.32^{b}	5.25±6.45^{d}	1.84
BLUT-4	21.53±0.46^{a}	288.75±6.76^{a}	293.75±7.03^{ab}	6.00±4.73^{d}	2.07
BMLT-1	22.74±1.28^{a}	287.75±10.16^{a}	302.25±3.62^{a}	14.50±9.12^{b}	5.03
BMLT-2	22.32±1.04^{a}	285.00±8.10^{a}	297.50±3.32^{ab}	12.25±8.88^{c}	4.29
BMLT-4	21.54±1.31^{a}	289.25±3.03^{a}	300.75±4.80^{a}	11.50±8.88^{c}	3.97

Values are Means±SEM, n = 5, ^{abc}Means in a column with different letters differ ($p\leq0.05$) using Tukey's studentized range test, BLUT: Blueberry leaf teas and BMLT: Bitter melon leaf tea

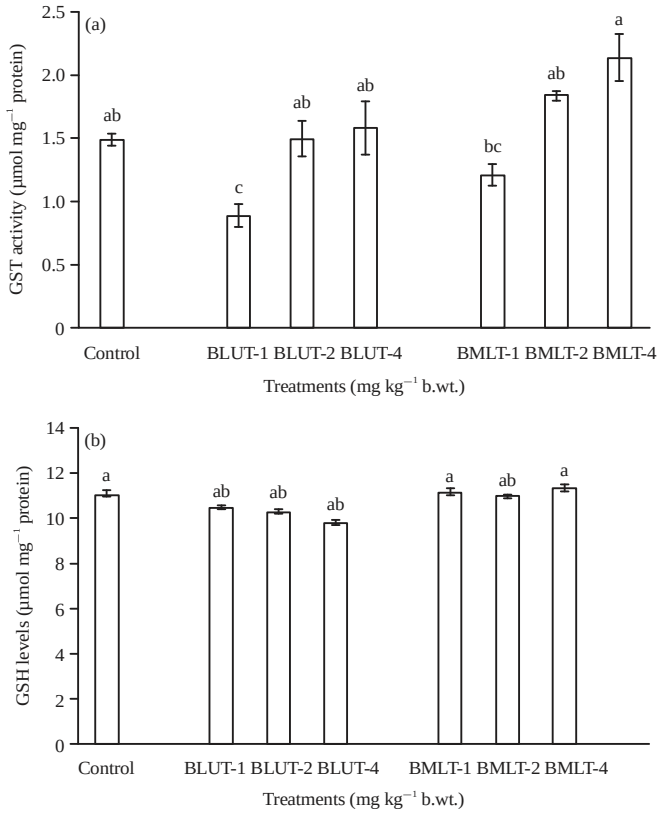

Fig. 1(a-b): Effect of Herbal leaf tea (Blue berry leaf (BLUT) and bitter melon leaf (BMLT)) administration on (a) Glutathione S- transferase activity and (b) Glutathione levels, values are±SEM n = 5, ^{ab}Mean on bars with different letters differ ($p \leq .05$) using Tukey's studentized range test. BLUT: Blueberry leaf tea, BMLT: Bitter melon leaf tea, GSH: Glutathione, GST: Glutathione-S-transferase

Table 2: Final weight and relative organ weights (RWs) of rats following sub-acute administration of herbal leafy teas (Blue berry leaf (BLUT) and bitter melon leaf (BMLT))

Groups	Body weight (final)	Liver	Kidney	Spleen	Heart	Testes	Brain	Thymus	Bone	Lung
Control	305.50^{a}	8.77^{a}	2.40	0.58	0.98	2.75	1.76^{ab}	0.25	0.93^{a}	1.70^{b}
BLUT-1	293.50^{ab}	8.23^{a}	2.30	0.65	1.15	2.88	1.73^{ab}	0.25	0.83^{a}	1.68^{b}
BLUT-2	289.75^{b}	7.82^{ab}	2.20	0.58	0.90	2.83	1.80^{a}	0.33	0.75^{a}	1.45^{c}
BLUT-4	293.75^{ab}	7.91^{ab}	2.36	0.60	0.96	2.75	1.83^{a}	0.25	0.75^{a}	1.65^{b}
BMLT-1	302.25^{a}	7.71^{ab}	2.28	0.64	1.00	2.73	1.77^{ab}	0.30	0.85^{a}	2.30^{a}
BMLT-2	297.75^{ab}	7.94^{ab}	2.18	0.60	1.02	2.60	1.73^{ab}	0.23	0.75^{a}	1.95^{ab}
BMLT-4	300.75^{a}	7.61^{b}	2.22	0.56	0.98	2.68	1.60^{b}	0.20	0.75^{a}	1.73^{b}

Values are ±SEM, n = 5, ^{abc}Mean in a column with different letters differ ($p \leq .05$) using Tukey's studentized range test, BLUT: Blueberry leaf tea and BMLT: Bitter melon leaf tea

exception of the group administered BMLT -4 (48% more than the control) and the BLUT-1 (40% less than the control). Among the treated groups, maximum induction in GST activity was noted in groups given BLMT-4 (2.14) and minimum induction in GST activity was observed in BLUT-1 (0.89). An increase in GST activity was noted with increasing dose for all the treatments administered.

Glutathione levels: Glutathione (GSH) is a tripeptide thiol present in high concentrations in most living cells. The GSH is involved in several crucial cellular reactions that protect cells from potentially toxic electrophiles formed through the metabolism of xenobiotics and these reactions have long been associated with the process of detoxification (Monks *et al*., 1990). In the current data (Fig. 1) no significant alterations in the GSH (µmol mg^{-1} protein) levels were observed. The GSH levels in the control untreated group did not significantly differ compared to the treatment groups. In effect, there were no significant ($p \leq 0.05$) differences in GSH levels among the treated groups or within the groups. Clearly, in this regard, administration of BMLT and BLUT did not affect GSH levels.

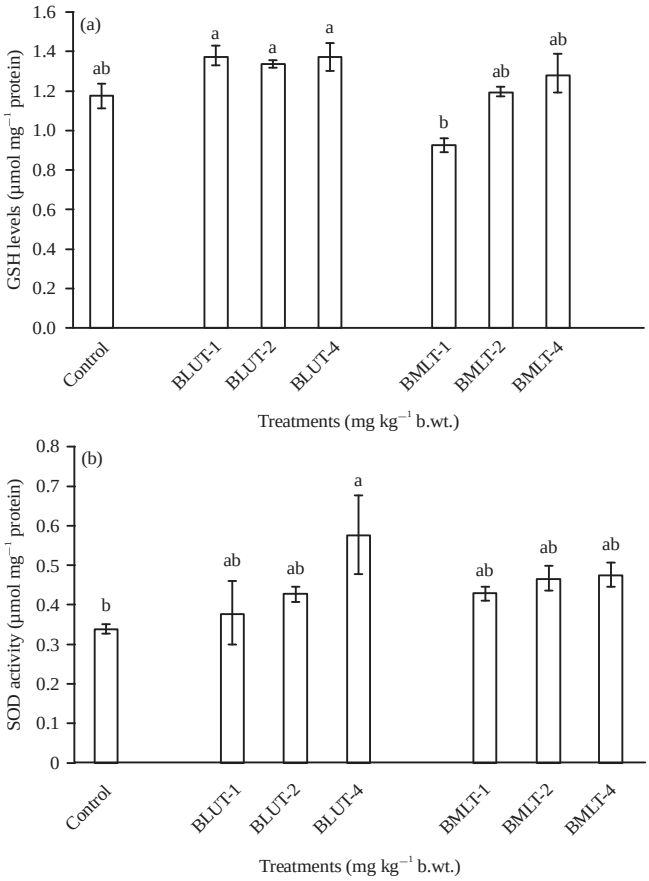

Fig. 2(a-b): Effect of leafy herbal teas (Blueberry leaf (BLUT) and bitter melon leaf (BMLT) administration on (a) Catalase (CAT) and (b) Superoxide dismutase (SOD) activity in rats, Values are ±SEM n = 5, [ab]Mean on bars with different letters differ ($p \leq .05$) using Tukey's studentized range test. BLUT: Blueberry leaf tea, BMLT: Bitter melon leaf tea.

Table 3: Total phenolic content, total flavonoid content and ferric reducing antioxidant potential in herbal leaf tea infusions

Groups	TF (mg CE/100 mL HLT)	TP (mg GAE/100 mL HLT)	FRAP (µmol Fe^{+2}/100 mL HLT)
BLUT	337.23±0.15[a]	88.41±0.29[a]	1137.06±0.45[a]
BMLT	26.47±0.03[b]	39.34±0.013[b]	1133.72±0.62[a]

Values are ±SEM (n = 6), [ab]Mean in column with different letters differ ($p \leq .05$) using Tukey's studentized range test. BLUT: Blueberry leaf tea, BMLT: Bitter melon leaf tea, CE: Catechin equivalents, GAE: Gallic acid equivalents, FRAP: Ferric reducing antioxidant power, HLT: Herbal leaf tea, TP: Total phenolic content, TF: Total flavonoid content

Catalase (CAT) activity: Catalase (CAT) (µmol mg^{-1} protein) activity are shown in Fig. 2a. Results indicated there were no significant differences in the treated groups and the control. The enzyme activities of the three doses of BLUT and BMLT (1, 2 and 4 mg kg^{-1} b.wt.) showed no significant differences ($p \leq 0.05$) compared to one another.

Superoxide dismutase (SOD) activity: The results of superoxide dismutase (SOD) (µmol mg^{-1} protein) activity in rats administered sub-acute doses of herbal leafy teas rats are shown in Fig. 2b. There was no significant difference in the activity of SOD between the CON and treated groups, except in the group administered BLUT-4. The SOD activity was significantly ($p \leq 0.05$) higher (approximately 41% increase) compared to CON. There were no significant differences among the treatment groups.

Total phenolic content, flavonoid content and ferric reducing antioxidant power in herbal leaf teas: Table 3 shows Total Flavonoid (TF) and Total Phenolic (TP) content of BLUT was significantly ($p<0.5$) higher compared to BMLT. The TF content in BLUT was approximately 10 times higher than in BMLT, while TP content was approximately two times higher. There was however, no significant difference with FRAP.

DISCUSSION

The present study aimed to evaluate the effects of a repeated 28-dayoral dose toxicity study of herbal leaf teas (bitter melon and blueberry) on liver function and endogenous antioxidant liver enzymes. Results showed that administration of herbal teas posed no adverse effects to the rats used in the study. The present study showed that rats administered herbal leaf teas, except for the BMLT group, amassed significantly ($p \leq 0.05$) lower weight gains compared to the control. Some studies have showed that bitter melon reduced body weight in experimental rodents (Chen and Li, 2005; Chao *et al*., 2011; Meng *et al*., 2012; Xu *et al*., 2014), most likely due to its effects on glucose metabolism. The current study is in agreement. Prior studies by Chen and Li (2005) indicated that chronic supplementation of bitter melon in a high fat diet slows weight gain and improves insulin sensitivity in rats fed an HF diet. The benefits ascribed to these effects are likely related to bioactive compound present in Bitter Melon (BM). In the BLUT supplemented groups, higher feed intakes resulted in significant ($p \leq 0.05$) reductions in body weight. This inverse association could be due to the thermogenic properties of polyphenols such as quercetin which some studies have shown stimulates brown adipose tissue thermogenesis and increases energy expenditure (Dulloo *et al*., 2000). Ahn *et al*. (2008), Lasa *et al*. (2012) and Park *et al*. (2008), went further to indicate that quercetin, in addition with other natural compounds such as resveratrol and catechin, effectively impacted weight loss and suppressed adipogenesis by inhibiting lipid accumulation in 3T3-L1 preadipocytes and primary human adipocytes.

While, these results were seen with the consumption of whole bitter melon and blueberry fruits, the present study indicate results from their herbal leaf infusions. In this study (Table 3), BLUT was found to contain high amount of total flavonoids and phenolics content. A study by Piljac-Zegarac *et al*. (2009), also reported high TP content and FRAP for wild blueberry infusion. Elsewhere, Zhu *et al*. (2013) showed that TFC and TPC from blueberries leaves were 114.21 and 425.24 mg g^{-1}, respectively. Results also indicate that BMLT is a rich source of polyphenols. Zhang *et al*. (2009) found that the major flavonoids and phenolic acids in bitter melon leaf were rutin, gentistic acid and o-coumaric acid. The BMLT exhibited a high FRAP which was comparable to BLUT. A study by Kubola and Siriamornpun (2008), noted that the bitter melon leaf had the highest FRAP compared to the fruit and stem. Taken together, these results indicate BLUT and BMLT are a good source of dietary phenolics and antioxidants with potential use in functional food products.

The effect of herbal leaf teas did not induce any significant ($p \leq 0.05$) alteration on hepatic GST activity when compared to the control (except in group BLUT-1). While there are no studies to our knowledge, on the effects of blueberry and bitter melon leaf teas on GST activity, this study can only attribute these effects on their bioactive contents. A study by Raza *et al*. (1996) showed that bitter melon juice stabilized GST activity by 30% in non-diabetic rats fed bitter melon juice compared to controls. Chou *et al*. (2000) also noted that polyphenols such as quercetin, catechin, anthocyanins, etc., in blueberries were found to participate in the modulation of GST activity. In another study, Dulebohn *et al*. (2008) found no significant increase in GST activity with blueberry supplementation in healthy rats. One of the known activities of phytochemicals is the induction of phase II metabolizing enzymes such as glutathione S-transferase (GST). The GSTs are needed by the liver to catalyze the conjugation of xenobiotics with GSH and are a vital part of the phase II detoxifying system. The liver is the primary organ exposed to the damaging effects of newly formed toxic, for that reason, protective mechanisms relevant to the liver are of particular interest. It is important to note that no significant adverse effects when compared to the control (untreated group) were observed with liver mass in the present study.

While, numerous studies to determine the *in vivo* antioxidant have been mostly directed towards green and black teas (*Camellia sinensis*) and their flowering parts, the present study highlight the effect of bitter melon leaf and blueberry leaf teas on glutathione (GSH) levels. The results showed that GSH levels in liver were not affected in rats administered leafy herbal teas at all doses. In fact, there was no significant difference in GSH levels when compared to the control (untreated). The maintenance of GSH levels is crucial, since GSH, a tripeptide molecule is known to play an important role in the detoxification of xenobiotics. This master antioxidant functions in the removal of potentially toxic electrophiles by maintaining cellular redox status. Depletion of GSH levels have been correlated to the development of irreversible cellular damage due to a sustained increase in cytosolic Ca^{+} levels (Miccadei *et al*., 1988; Martensson and Meister, 1989; Castell *et al*., 1997; Lima *et al*., 2006). Thus, increases in GSH levels which were observed in the present study, are expected to reduce ROS levels and antagonize toxicity events (Du *et al*., 2009). The results from this study are in line with an animal study conducted by Asiamah *et al*. (2011). In that study, rats fed a diet of bitter melon had significantly ($p \leq 0.05$) high GSH levels compared to the control. It was concluded that bitter melon has antioxidative properties including the enhancement of antioxidative

enzymes and compounds (Asiamah *et al*., 2011). The protective properties of blueberries on hepatic function have been adequately reported. Earlier, Coban *et al*. (2013) showed that hepatic GSH levels remained unchanged in hypocholestereolemic guinea pigs fed powdered blueberries. Blueberry supplementation was also found to increase GSH levels and reduce liver injury in Diethylnitrosamine (DEN) induced rats (Bingul *et al*., 2013). Based on the present results, the hepatoprotective effects of sub-acute administration of BLUT are in part attributed to their antioxidative properties, which in turn significantly increased the antioxidative capacity of the liver.

The SOD and catalase activities were also not affected after supplementation with herbal leaf teas. It has been indicated that the presence of antioxidants such as catechin improves endogenous antioxidant enzymes including SOD, Catalase and GPx (Sohn *et al*., 1994). As indicated earlier, although studies depicting the benefits of blueberry leaf and bitter melon leaf tea consumption are scarce, elsewhere, blueberry juice consumption was reported to significantly increase SOD activity in male Sprague-Dawley rats (Wang *et al*., 2013). The authors pointed out that the protective effect of blueberry is perhaps through inhibition of liver inflammation. In another study, oral treatment with *Camellia sinensis* extractsin rats restored CAT and SOD activities to normal levels after previous exposure to carbon tetrachloride (CCl_4) (Sengottuvelu *et al*., 2008). Catalase (CAT) and superoxide dismutase (SOD) are important determinants of the cellular antioxidant system and because these enzymes act sequentially to remove Reactive Oxygen Species (ROS), the balance of the activity of these enzymes may be as crucial in the defense against ROS as the activity of the enzymes alone. As previously pointed out by Loew and Kaszkin (2004) and further expanded on, Abolfathi *et al*. (2012) implied that in general herbal medicines are complex mixtures of different compounds that often act in a synergistic fashion and exert their full beneficial effect as total extracts.

CONCLUSION

The observations from this study indicate that a repeated 28-dayoral supplementation with bitter melon leaf and blueberry leaf teas maintained the activity and levels of endogenous antioxidant enzymes. The recovery of the redox system prevented oxidative stress induced liver injury. Additionally the present investigation demonstrates that at the doses consumed, bitter melon leaf and blueberry leaf teas may be considered relatively safe, as it did not cause any lethality or changes in the general behavior in this study. In the future, we will determine (1) if such effects on liver function and endogenous antioxidant liver enzymes could be observed after prolonged consumption of bitter melon leaf and blueberry leaf teas and (2) bioaccessibility and bioavailability of the BLUT and BMLT polyphenols.

REFERENCES

Abolfathi, A.A., D. Mohajeri, A. Rezaie and M. Nazeri, 2012. Protective effects of green tea extract against hepatic tissue injury in streptozotocin-induced diabetic rats. Evidence-Based Complement. Altern. Med. 10.1155/2012/740671

Ahn, J., H. Lee, S. Kim, J. Park and T. Ha, 2008. The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. Biochem. Biophys. Res. Commun., 373: 545-549.

Asiamah, D., M. Verghese, J. Boateng, B. Kanda, L. Shackelford and L.T. Walker, 2011. Chemopreventive potential of bitter melon (*Momordica charantia*) against precancerous lesions in the colon of fisher 344 male rats. Int. J. Cancer Res., 7: 36-46.

Barnes, J., 2003. Quality, efficacy and safety of complementary medicines: Fashions, facts and the future. Part II: Efficacy and safety. Br. J. Clin. Pharmacol., 55: 331-340.

Barnes, J.O., 2002. An introduction to herbal medicinal products. Pharm. J., 268: 804-808.

Benzie, I.F.F. and J.J. Strain, 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Method Enzymol., 299: 15-27.

Bingul, I., C. Basaran-Kucukgergin, M.S. Tekkesin, V. Olgac, S. Dogru-Abbasoglu and M. Uysal, 2013. Effect of blueberry pretreatment on diethylnitrosamine-induced oxidative stress and liver injury in rats. Environ. Toxicol. Pharmacol., 36: 529-538.

Bun, S.S., H. Bun, D. Guedon, C. Rosier and E. Ollivier, 2006. Effect of green tea extracts on liver functions in Wistar rats. Food Chem. Toxicol., 44: 1108-1113.

Castell, J.V., M.J. Gomez-Lechon, X. Ponsoda and R. Bort, 1997. The use of cultured hepatocytes to investigate the mechanisms of drug hepatotoxicity. Cell Biol. Toxicol., 13: 331-338.

Chang, Z.G., D.T. Kennedy, D.A. Holdford and R.E. Small, 2007. Pharmacists' knowledge and attitudes toward herbal medicine. Ann. Pharmacother., 41: 1272-1276.

Chao, C. Y., M.C. Yin and C.J. Huang, 2011. Wild bitter gourd extract up-regulates mRNA expression of PPARα, PPARγ and their target genes in C57BL/6J mice. J. Ethnopharmacol., 135: 156-161.

Chen, Q. and E.T.S. Li, 2005. Reduced adiposity in bitter melon (*Momordica charantia*) fed rats is associated with lower tissue triglyceride and higher plasma catecholamines. Br. J. Nutr., 93: 747-754.

Chou, F.P., Y.C. Chu, J.D. Hsu, H.C. Chiang and C.J. Wang, 2000. Specific induction of glutathione-S-transferase GSTM2 subunit expression by epigallocatechin gallate in rat liver. Biochem. Pharmacol., 60: 643-650.

Coban, J., B. Evran, F. Ozkan, A. Cevik, S. Dogru-Abbasoglu and M. Uysal, 2013. Effect of blueberry feeding on lipids and oxidative stress in the serum, liver and aorta of guinea pigs fed on a high-cholesterol diet. Biosci. Biotechnol. Biochem., 77: 389-391.

Critchley, L.A.H., D.Q. Chen, A. Lee, G.N. Thomas and B. Tomlinson, 2005. A survey of Chinese herbal medicine intake amongst preoperative patients in Hong Kong. Anaesthesia Intensive Care, 33: 506-513.

Cupp, M.J., 1999. Herbal remedies: Adverse effects and drug interactions. Am. Family Phys., 59: 1239-1245.

Dasgupta, A., 2010. Herbal toxicity. Med. Lab. Observer, 42: 24-24.

Deng, J.F., 2002. Clinical and laboratory investigations in herbal poisonings. Toxicology, 181-182: 571-576.

DiPaola, R.S., H. Zhang, G.H. Lambert, R. Meeker and E. Licitra *et al*., 1998. Clinical and biologic activity of an estrogenic herbal combination (PC-SPES) in prostate cancer. N. Engl. J. Med., 339: 785-791.

Du, Z.X., H.Y. Zhang, X. Meng, Y. Guan and H.Q. Wang, 2009. Role of oxidative stress and intracellular glutathione in the sensitivity to apoptosis induced by proteasome inhibitor in thyroid cancer cells. BMC Cancer, Vol. 9. 10.1186/1471-2407-9-56.

Dulebohn, R.V., W. Yi, A. Srivastava, C.C. Akoh, G. Krewer and J.G. Fischer, 2008. Effects of blueberry (*Vaccinium ashei*) on DNA damage, lipid peroxidation and phase II enzyme activities in rats. J. Agric. Food Chem., 56: 11700-11706.

Dulloo, A.G., J. Seydoux, L. Girardier, P. Chantre and J. Vandermander, 2000. Green tea and thermogenesis: Interactions between catechin-polyphenols, caffeine and sympathetic activity. Int. J. Obesity, 24: 252-258.

Fernandez-Urrusuno, R., E. Fattal, J. Feger, P. Couvreur and P. Therond, 1997. Evaluation of hepatic antioxidant systems after intravenous administration of polymeric nanoparticles. Biomaterials, 18: 511-517.

Gajula, D., M. Verghese, J. Boateng, L.T. Walker, L. Shackelford, S.R. Mentreddy and S. Cedric, 2009. Determination of total phenolics, flavonoids and antioxidant and chemopreventive potential of basil (*Ocimum basilicum* L. and *Ocimum tenuiflorum* L.). Int. J. Cancer Res., 5: 130-143.

Griffith, O.W., 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem., 106: 207-212.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Halsted, C.H., 2003. Dietary supplements and functional foods: 2 sides of a coin. Am. J. Clin. Nutr., 77: 1001S-1007S.

Issa, A.Y., S.R. Volate and M.J. Wargovich, 2006. The role of phytochemicals in inhibition of cancer and inflammation: New directions and perspectives. J. Food Compos. Anal., 19: 405-419.

Johansson, L.H. and L.A. Borg, 1988. A spectrophotometric method for determination of catalase activity in small tissue samples. Anal. Biochem., 174: 331-336.

Kubola, J. and S. Siriamornpun, 2008. Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts *in vitro*. Food Chem., 110: 881-890.

Lasa, A., I. Churruca, I. Eseberri, C. Andres-Lacueva and M.P. Portillo, 2012. Delipidating effect of resveratrol metabolites in 3T3-L1 adipocytes. Mol. Nutr. Food Res., 56: 1559-1568.

Lima, C.F., M. Fernandes-Ferreira and C. Pereira-Wilson, 2006. Phenolic compounds protect HepG2 cells from oxidative damage: Relevance of glutathione levels. Life Sci., 79: 2056-2068.

Loew, D. and M. Kaszkin, 2002. Approaching the problem of bioequivalence of herbal medicinal products. Phytother. Res., 16: 705-711.

Mahady, G.B., 2001. Global harmonization of herbal health claims. J. Nutr., 131: 1120S-1123S.

Martensson, J. and A. Meister, 1989. Mitochondrial damage in muscle occurs after marked depletion of glutathione and is prevented by giving glutathione monoester. Proc. Natl. Acad. Sci., 86: 471-475.

Mehta, D.H., R.S. Phillips, R.B. Davis and E.P. McCarthy, 2007. Use of complementary and alternative therapies by Asian Americans. Results from the national health interview survey. J. Gen. Internal Med., 22: 762-767.

Meng, Y., B. Liu, N. Lei, J. Zheng and Q. He *et al*., 2012. Alpha-momorcharin possessing high immunogenicity, immunotoxicity and hepatotoxicity in SD rats. J. Ethnopharmacol., 139: 590-598.

Miccadei, S., D. Nakae, M.E. Kyle, D. Gilfor and J.L. Farber, 1988. Oxidative cell injury in the killing of cultured hepatocytes by allyl alcohol. Arch. Biochem. Biophys., 265: 302-310.

Monks, T.J., M.W. Anders, W. Dekant, J.L. Stevens, S.S. Lau and P.J. van Bladeren, 1990. Glutathione conjugate mediated toxicities. Toxicol. Applied Pharmacol., 106: 1-19.

Park, H.J., J.Y. Yang, S. Ambati, M.A. Della-Fera, D.B. Hausman, S. Rayalam and C.A. Baile, 2008. Combined effects of genistein, quercetin and resveratrol in human and 3T3-L1 adipocytes. J. Med. Food, 11: 773-783.

Piljac-Zegarac, J., A. Belscak and A. Piljac, 2009. Antioxidant capacity and polyphenolic content of blueberry (*Vaccinium corymbosum* L.) leaf infusions. J. Med. Food, 12: 608-614.

Raza, H., I. Ahmed, M.S. Lakhani, A.K. Sharma, D. Pallot and W. Montague, 1996. Effect of bitter melon (*Momordica charantia*) fruit juice on the hepatic cytochrome P450-dependent monooxygenases and glutathione S-transferases in streptozotocin-induced diabetic rats. Biochem. Pharmacol., 52: 1639-1642.

Raza, H., I. Ahmed, A. John and A.K. Sharma, 2000. Modulation of xenobiotic metabolism and oxidative stress in chronic streptozotocin-induced diabetic rats fed with *Momordica charantia* fruit extract. J. Biochem. Mol. Toxicol., 14: 131-139.

Reeves, P.G., F.H. Nielsen and G.C. Fahey Jr., 1993a. AIN-93 purified diets for laboratory rodents: Final report of the American institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.

Reeves, P.G., K.L. Rossow and J. Lindlauf, 1993b. Development and testing of the AIN-93 purified diets for rodents: Results on growth, kidney calcification and bone mineralization in rats and mice. J. Nutr., 123: 1923-1931.

Ridker, P.M., 1987. Toxic effects of herbal teas. Arch. Environ. Health, 42: 133-136.

SAS., 2013. Statistical Analysis System User Guide, Version 9.2. SAS Institute Inc., Cary, NC., USA.

Schoepfer, A.M., A. Engel, K. Fattinger, U.A. Marbet and D. Criblez *et al*., 2007. Herbal does not mean innocuous: Ten cases of severe hepatotoxicity associated with dietary supplements from Herbalife® products. J. Hepatol., 47: 521-526.

Sengottuvelu, S., S. Duraisami, J. Nandhakumar, R. Duraisami and M. Vasudevan, 2008. Hepatoprotective activity of *Camellia sinensis* and its possible mechanism of action. Iran. J. Pharmacol. Therapeut., 7: 9-14.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Metho. Enzymol., 299: 152-178.

Sohn, O.S., A. Surace, E.S. Fiala, J.P. Richie, S. Colosimo, E. Zang and J.H. Weisburger, 1994. Effects of green and black tea on hepatic xenobiotic metabolizing systems in the male F344 rat. Xenobiotica, 24: 119-127.

Sydara, K., S. Gneunphonsavath, R. Wahlstrom, S. Freudenthal, K. Houamboun, G. Tomson and T. Falkenberg, 2005. Use of traditional medicine in Lao PDR. Complement. Therapies Med., 13: 199-205.

WHO., 2008. Traditional medicine. WHO Fact Sheet No. 134. http://www.who.int/mediacentre/factsheets/2003/fs134/en/

Wang, Y., M. Cheng, B. Zhang, F. Nie and H. Jiang, 2013. Dietary supplementation of blueberry juice enhances hepatic expression of metallothionein and attenuates liver fibrosis in rats. PloS ONE, Vol. 8. 10.1371/journal.pone.0058659.

Xu, J., K. Cao, Y. Li, X. Zou and C. Chen *et al*., 2014. Bitter gourd inhibits the development of obesity-associated fatty liver in C57BL/6 mice fed a high-fat diet. J. Nutr., 144: 475-483.

Zhang, M., N.S. Hettiarachchy, R. Horax, P. Chen and K.F. Over, 2009. Effect of maturity stages and drying methods on the retention of selected nutrients and phytochemicals in bitter melon (*Momordica charantia*) leaf. J. Food Sci., 74: C441-C448.

Zhao, J., J.W. Deng, Y.W. Chen and S.P. Li, 2013. Advanced phytochemical analysis of herbal tea in China. J. Chromatogr. A, 1313: 2-23.

Zhishen, J., T. Mengcheng and W. Jianming, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.

Zhu, L., X. Liu, J. Tan and B. Wang, 2013. Influence of harvest season on antioxidant activity and constituents of rabbiteye blueberry (*Vaccinium ashei*) leaves. J. Agric. Food Chem., 61: 11477-11483.