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Hypolipemic and Weight Reducing Properties from *Tamarindus indica* L. Pulp Extract in Diet-Induced Obese Rats

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Abstract: The global prevalence of overweight and obesity has reached epidemic proportions. Hyperlipemia couple with increased oxidative stress generates various degenerative diseases such as hypertension and cardiovascular problems. In the present study, a hypolipemic and weight reducing effects of crude *Tamarindus indica* L. pulp extract were examined in adult Sprague-Dawley rats fed a high-fat diet. Animals were fed on either normal chow or high-fat diet for 10 weeks for obesity induction and subsequently received either placebo or *T. indica* L. extract at 5, 25, 50 or 300 mg kg^{-1} chitosan via oral gavage for another 10 weeks. Treatment of obese rats with the *T. indica* pulp extract led to a decrease in the levels of plasma total cholesterol (TCHOL), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) and increase high-density lipoprotein cholesterol (HDL-C) level with concomitant reduction of body weight. The extract improved the efficiency of the antioxidant defense system, as indicated by increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and subsequently resulted in significantly lower lipid peroxidation indices; malondialdehyde (MDA) level. Together these results indicate the potential use of *T. indica* extracts as hypolipemic and antioxidative agent apart from its ability to reduce body weight in obese-induced rats.

Key words: Hypolipemia, weight maintenance, free radical, lipid peroxidation, antioxidant, tamarind

INTRODUCTION

Obesity is common nutritional disorder in the industrialized world and it is considered to be a risk factor associated with the development of the major human health problems, including cardiovascular diseases and diabetes. Increased fat consumption have been associated with the risk of obesity and hyperlipidemia in human and rodents via alteration of cholesterol and triglyceride levels in plasma and tissues (Bray, 2004; Estadella *et al*., 2004). On the other hand, elevated levels of plasma low density lipoprotein cholesterol (LDL-C) and triglycerides, accompanied by reduced high density lipoprotein cholesterol (HDL-C) levels, is often associated with an increased risk of CVD. Of particular importance, patients with cardiovascular and cerebrovascular diseases have been reported to consumed diet with more fat content than the general population (Bowen and Borthakur, 2004), suggesting that high-fat diet may accelerate the development of obesity and heart problems. Fardet *et al.* (2008) reported that the diet-induced obesity in rat models showed an increased level of oxidative stress in their liver and that oxidative stress can result from the excessive production of reactive oxygen species and/or deficient antioxidant capacity. Thus, dietary supplementation possesses antioxidant and lipid lowering effects has been proposed as a potential therapeutic approach to prevent and manage obesity and its associated complications.

Tamarindus indica is a perennial herb belonging to the dicotyledonous family of Leguminosae. Its local names include Indian date (English), asam jawa (Malay), siyambala (Sinhala), sampalog (Philippines) and puli (Tamil). The tree averages 20-25 m in height and 1 m in diameter, it has a wide spreading crown and a short, stout trunk.

The pulp is widely consumed in many countries for seasoning, as a food component and in juices owing to its

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desirable taste. Its fruit is regarded traditionally as a digestive, carminative, laxative, expectorant and blood tonic (Komutarin *et al.*, 2004). Other parts of the plant present antioxidant (Joyeux *et al.*, 1995), antihepatotoxic (Maiti *et al.*, 2004), antiinflammatory (Ramos *et al.*, 2003), antimutagenic (Rimbau *et al.*, 1999) and antidiabetic activities (Tsuda *et al.*, 1994). Despite its popularity amongst traditional healers in the treatment of diseases, the antiobesity and lipid lowering potential of *T. indica* fruit remained to be defined. Therefore, the aim of the present study was to determine the effect of *T. indica* pulp extract as antiobesity and a lipid-lowering agent in rat model. In addition, assessment of lipid peroxidation effect of the extract is also being carried out to determine its antioxidative status in rats induced obesity. We hypothesized that the *T. indica* extract may contain dietary antioxidants that improve blood lipids levels. The antiobesity effect of the *T. indica* extract in rats fed a high-fat diet may be partly due to the inhibition of intestinal absorption of dietary fat by the high digestible starch and acidic content of *T. indica*.

MATERIALS AND METHODS

This study was carried out from March 2008 to February 2009 at the Animal House Unit, Faculty of Medicine and Health Sciences and Pathology Laboratory, Universiti Putra Malaysia (UPM).

Chemical reagents: Trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), 1, 1, 3, 3-tetraetoxypropane (TEP), n-butanol, chitosan C3646 and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (USA). Diethyl ether and absolute ethanol were obtained from Merck (Germany). Sodium hydroxide (NaOH), copper (II) sulfate ($CuSO_4$), potassium sodium tartarate ($C_4H_4KNaO_6 \cdot 4H_2O$) were purchased from Fluka (Switzerland). All chemicals were of analytical grade.

Preparation of *Tamarindus indica* pulp aqueous extract: Fresh matured tamarind fruits were collected from Universiti Putra Malaysia (UPM) herbal center after being identified and confirmed by a plant taxonomist. The voucher number of the plant is SK15662 and being deposited in the Herbarium Unit, UPM. The fruits were peeled and the pulps were separated from the seed. Ten percent aqueous pulp extract was prepared by soaking 100 g of the fresh pulp (equivalent to 25 tamarind pods) in 1L of distilled water and mixed thoroughly. The mixture was boiled in shaking water bath at 100°C for 15 min. Once filtered, the filtrates were spray dried subsequently at inlet and outlet temperatures of 150 and 80°C, respectively. The prepared extracts were kept at -20°C until used.

Induction of experimental obese rats: Forty two rats at the age of 3 months, weighing between 200 to 250 g were acclimatized under room temperature (28±2°C) with regular light/dark cycle and free access to food and water for one week before use. Following acclimatization, the animals were randomly segregated into six groups of seven rats each. Five groups were given high-fat diet. Another one group noted as Normal Control (NC) was given normal rat chow purchased from Miba Mansura Trading, Malaysia. The high-fat diet was formulated based on the composition provided by Levin and Ambrose (2002). The diet consist a mixture of 50% normal rat chow pellet (Miba Mansura Trading, Malaysia), 24% corn oil (Mazola), 20% full-cream milk powder (Nespray, Nestlé) and 6% sugar. All ingredients were thoroughly mixed and baked in the oven at 40°C overnight. The food prepared was cut into smaller pieces before given to the rats. Water and both types of diet were given *ad libitum* for 10 weeks. After 10 weeks, the mean body weights of the high-fat diet treated groups were compared to that of the NC group. The groups with significantly higher mean body weights than the NC group were considered as obese (Levin and Ambrose, 2002). The experiment protocol and animal handling throughout the study are in accordance with guideline approved by the institution ethics committee where the study is conducted.

Supplementations of *Tamarindus indica* extract and chitosan: Treatment began after 10 weeks of obesity induction and labeled as week 0. *Tamarindus indica* extract supplementation were given at three different doses; 5, 25 and 50 mg kg^{-1}. The respective extracts were prepared by adding 0.5, 2.5 and 5 g of spray-dried *T. indica* extract to 100 mL of distilled water. Chitosan was given at 300 mg kg^{-1} rat weight where 30 g of chitosan was dissolved to 100 mL of distilled water to obtain a concentration of 300 mg kg^{-1}, each in 0.1 mL volume. The supplementations were given once daily to the animals via oral gavage for 10 weeks, at a volume of 0.1 mL/100 g body weight, while Normal Control (NC) and Positive Control (PC) received distilled water as placebo for 10 weeks. Contrary from the induction period, all groups were given normal rat chow during this supplementation period (Table 1).

Table 1: Depiction of each group and its respective treatment

Groups	Description
NC	Normal+distilled water
PC	Obese+distilled water
T1	Obese+5 mg kg^{-1} *Tamarindus indica* extract
T2	Obese+25 mg kg^{-1} *Tamarindus indica* extract
T3	Obese+50 mg kg^{-1} *Tamarindus indica* extract
Chi	Obese+300 mg kg^{-1} chitosan

Blood sampling: Blood sampling was performed three times; before the obesity induction begun (week -10), after the induction completed and before the treatment began (week 0) and after the treatment completed (week 10); to determine the effects of the *T. indica* extract and chitosan on the blood biochemical parameters in the experimental animals. Approximately 3 mL blood was collected into EDTA and lithium heparin tubes via cardiac puncture. The blood collected in the EDTA tube was centrifuged at 3000 rpm at 4°C for 10 min and the plasma was stored at -80°C. The blood collected in the heparinized tube was processed in the same day of blood collection for superoxide dismutase (SOD) and glutathione peroxidase (GPx) evaluation.

Bodyweight and food intake: Individual bodyweight and food intake were recorded weekly using electrical balance (AND, HR-200). Food intake was the amount of food consumed by each rat within 24 h.

Plasma lipid profile analysis: All plasma lipids were estimated spectrophotometrically at 600 nm using commercial rapid test kit (Roche Diagnostics). Total cholesterol (TCHOL) is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The resulting hydrogen peroxide substrates form a red dyestuff by reacting with 4-aminoantipyrine and phenol under the catalytic action of peroxidase. The color intensity was directly proportional to the concentration of cholesterol and was detected at 600 nm spectrophotometrically.

The HDL-C assay consists of two distinct reaction steps. The first step is the elimination of chylomicron, VLDL-C and LDL-C by cholesterol esterase, cholesterol oxidase and subsequently catalase. The second step is the specific measurement of HDL-C after the release of HDL-C by sodium azide detergents. The intensity of the quinoneimine dye produced is directly proportional to the HDL-C concentration when measured spectrophotometrically at 600 nm.

The LDL-C analysis is a homogenous enzymatic calorimetric assay based on the reaction between LDL-C and cholesterol esterase and cholesterol oxidase. The resulting hydrogen peroxide substrates form a red dyestuff by reacting with 4-aminoantipyrine and N-(2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyamiline (HDAOS) under the catalytic action of peroxidase. The color intensity was directly proportional to the concentration of LDL-C and was determined spectrophotometrically at 600 nm.

Triglycerides (TG) were determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The color intensity was directly proportional to the concentration of triglycerides and was detected at 600 nm spectrophotometrically.

Microsomal lipid peroxidation and protein estimation: Plasma samples obtained were used to study lipid peroxidation in vivo. Malondialdehyde (MDA) as thiobarbituric acid reactive substances was measured at 532 nm spectrophotometrically (Bolkent *et al*., 2005) whereas the protein concentration was determined according to the method of Kingsley (1942) with minor modification.

Superoxide dismutase (SOD) level determination: The activity of superoxide dismutase (SOD) was assayed by the RANSOD (RANDOX Laboratories Ltd., USA) kit. The samples used were heparinized whole blood. The role of SOD was to accelerate the dismutation of the toxic superoxide radical ($O_2^{\bullet}$) produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay.

The 0.5 mL heparinized whole blood sample was transferred to Selektra-E tube and centrifuged at 3000 rpm for 10 min. The plasma was then aspirated off. The erythrocytes were washed four times with 3 mL of normal saline (0.9% NaCl) solution centrifuging for 10 min at 3000 rpm after each wash. Subsequently, the washed centrifuged erythrocytes was made up to 2 mL with cold distilled water, mixed and left to stand at 4°C for 15 min to form lysate. The absorbance was read spectrophotometrically at 505 nm via clinical chemistry analyzer machine (Vitalab Selektra E, Germany).

Glutathione peroxidase (GPx) level determination: The activity of glutathione peroxidase (GPx) was assayed by the RANSEL (RANDOX Laboratories Ltd., USA) kit, using heparinized whole blood according to method suggested by Paglia and Valentine (1967). GPx catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase

(GRx) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The 0.05 mL of heparinized whole blood sample was transferred into Selektra-E tube and further added with 2 mL of Ransel diluting agent. The mixture was mixed well and incubated for 5 min. Absorbance was then read at 340 nm via chemistry analyzer system (Vitalab Selektra E, Germany).

Statistical analysis: The data obtained were analyzed using the Statistical Package for Social Science (SPSS) version 14 programme. After confirming the normality of data and the homogeneity of variance of data, the significance of the differences between the means of the test and control studies was established by one-way Analysis of Variance (ANOVA) coupled with post hoc Tukey HSD test for multiple group comparison. A value of $p<0.05$ was used to denote statistical significance. Results are expressed as Mean±SE mean (SEM).

RESULTS

Food intake and body weight: Table 2 shows the mean of food and caloric intake of each group from week -10 to week 0 (obesity induction period) and from week 0 to week 10 (supplementation period). There was no significant difference of food intake observed for all groups during the induction period. However, the caloric intake between the normal diet treated group (NC) and the high-fat diet treated groups (PC, T1, T2, T3 and Chi) was significantly different ($p<0.05$). During the supplementation period, T2 and T3 groups showed significantly lower food and caloric intake than the PC and Chi groups.

Table 3 shows the mean of body weight and the body weight gain of the control groups and the treated groups. There was no significant difference of body weight between each group at week -10 ($p>0.05$). After received their respective diets, there was significant weight difference between the normal control group and all the obese induced groups ($p<0.05$). Since, all the high-fat diet treated groups had significantly higher mean body weight than the normal control group at week 0, they are all verified to be obese; hence proceeded to the supplementation phase.

There was still a significant difference existed ($p<0.05$) between the NC and PC groups after 10 weeks of supplementation period. Interestingly, the mean body weight and the body weight gain of T2, T3 and Chi groups were found to be significantly lower than the PC group. On the other hand, the body weight of the T1 group showed a significant difference to the NC group and showed no significant difference to the PC group.

Plasma lipids analysis: The effects of *T. indica* extracts and chitosan supplementation on plasma lipids levels are shown in Table 4. As expected, the plasma total cholesterol clearly increased in the groups treated with high-fat diet for 10 weeks (PC, T1, T2, T3 and Chi), when compared to the normal control (NC). Treatment of the obese groups with *T. indica* extracts (T1, T2 and T3) and chitosan (Chi) resulted in a significant decrease in the plasma total cholesterol levels, when compared to the PC group. A similar response was also observed for plasma LDL-C cholesterol levels. Furthermore, the treatment with *T. indica* extracts and chitosan resulted in a substantial increase in the plasma HDL-C levels when compared to the PC group. On the other hand, there was no significant difference in the plasma triglycerides levels between each group prior and after treatment.

In present study, *T. indica* extract and chitosan treated groups showing a low TCHOL:HDL ratio significantly at week 10 ($p<0.05$) compared to that of PC

Table 2: Food and caloric intake of control groups and obese groups treated with *T. indica* extract and chitosan

	Food intake (g/rat/day)		Caloric intake (kcal/g/rat/day)	
Groups	Week -10-0	Week 0-10	Week -10-0	Week 0-10
NC	19.57±0.44^a	24.43±1.95^{ab}	74.36±1.67^a	92.83±7.41^{ab}
PC	18.13±0.46^a	25.00±1.65^b	91.78±2.33^b	95.00±6.27^b
T1	18.35±0.54^a	23.45±1.75^{ab}	92.91±2.72^b	89.11±6.65^{ab}
T2	18.93±0.56^a	21.98±1.35^a	95.82±2.85^b	87.36±5.13^a
T3	18.16±0.85^a	21.67±1.27^a	91.93±4.30^b	82.76±4.83^a
Chi	18.67±0.68^a	25.46±1.27^b	94.53±3.44^b	96.75±4.83^b

Values represent the Mean±SEM (n = 7). Values for a given parameter in a column that do not share a common superscript are significantly different at $p<0.05$. NC: Normal control; PC: Positive control; T1: *T. indica* extract 5 mg kg^{-1}; T2: *T. indica* extract 25 mg kg^{-1}; T3: *T. indica* extract 50 mg kg^{-1}; Chi: Chitosan 300 mg kg^{-1}

Table 3: Mean of body weight of rats prior the obesity induction (Week -10), after the obesity induction and prior treatment (Week 0) and after treatment (Week 10)

	Body weight (g)					
Weeks	NC	PC	T1	T2	T3	Chi
-10	200.00±3.16^a	202.57±4.78^a	191.57±3.50^a	202.75±5.99^a	208.50±5.42^a	205.14±4.84^a
0	406.57±8.59^a	463.43±15.67^b	476.57±13.38^b	460.63±13.08^b	462.13±6.23^b	465.29±5.92^b
10	456.57±16.59^a	561.14±10.91^b	543.14±15.51^{bc}	497.63±16.25^{ac}	493.00±10.64^{ac}	491.71±7.80^{ac}

Values represent the mean±SEM (n = 7). Values for a given parameter in a row that do not share a common superscript are significantly different at $p<0.05$. NC: Normal control; PC: Positive control; T1: *T. indica* extract 5 mg kg^{-1}; T2: *T. indica* extract 25 mg kg^{-1}; T3: *T. indica* extract 50 mg kg^{-1}; Chi: Chitosan 300 mg kg^{-1}

Table 4: Plasma lipids and the ratio off TCHOL and HDL-C of rats prior the obesity induction (Week -10), after the obesity induction and prior treatment (Week 0) and after treatment (Week 10)

	NC	PC	T1	T2	T3	Chi
TCHOL (mmol L^{-1})						
Week -10	0.48±0.02$^{a\#}$	0.49±0.03$^{a\#}$	0.47±0.01$^{a\#}$	0.51±0.02$^{a\#}$	0.50±0.05$^{a\#}$	0.47±0.05$^{a\#}$
Week 0	1.20±0.04^{a*}	1.47±0.07^{b*}	1.47±0.04^{b*}	1.44±0.06^{b*}	1.47±0.05^{b*}	1.49±0.05^{b*}
Week 10	1.26±0.02^{a*}	1.37±0.02^{a*}	1.22±0.08$^{a*\#}$	1.22±0.05^{a*}	1.24±0.01$^{a*\#}$	1.28±0.07$^{a*\#}$
HDL-C (mmol L^{-1})						
Week -10	0.43±0.02$^{a\#}$	0.43±0.06$^{a\#}$	0.40±0.05$^{a\#}$	0.44±0.04$^{a\#}$	0.44±0.03$^{a\#}$	0.41±0.06$^{a\#}$
Week 0	0.77±0.03^{a*}	0.63±0.02^{b*}	0.64±0.02^{b*}	0.62±0.03^{b*}	0.63±0.02^{b*}	0.65±0.03^{b*}
Week 10	1.09±0.04$^{a*\#}$	0.89±0.03$^{b*\#}$	1.04±0.02$^{ab*\#}$	1.07±0.05$^{a*\#}$	1.08±0.06$^{a*\#}$	1.00±0.03$^{ab*\#}$
LDL-C (mmol L^{-1})						
Week -10	0.14±0.06$^{a\#}$	0.17±0.02$^{a\#}$	0.14±0.03$^{a\#}$	0.16±0.01$^{a\#}$	0.12±0.04$^{a\#}$	0.11±0.06$^{a\#}$
Week 0	0.18±0.01^{a*}	0.27±0.01^{b*}	0.27±0.03^{b*}	0.28±0.02^{b*}	0.26±0.01^{b*}	0.25±0.02^{b*}
Week 10	0.23±0.02^{a*}	0.30±0.01^{b*}	0.24±0.02a^{b*}	0.23±0.01a^{b*}	0.23±0.01^{a*}	0.24±0.02a^{b*}
TG (mmol L^{-1})						
Week -10	0.23±0.03$^{a\#}$	0.22±0.03$^{a\#}$	0.22±0.01$^{a\#}$	0.22±0.03$^{a\#}$	0.22±0.02$^{a\#}$	0.23±0.02$^{a\#}$
Week 0	0.31±0.02*	0.40±0.03*	0.38±0.03*	0.40±0.02*	0.38±0.02*	0.36±0.03*
Week 10	0.43±0.02*	0.50±0.02*	0.42±0.01*	0.45±0.02*	0.44±0.03*	0.46±0.02*
TCHOL:HDL ratio						
Week -10	1.10±0.02$^{a\#}$	1.15±0.02$^{a\#}$	1.17±0.02$^{a\#}$	1.18±0.02$^{a\#}$	1.14±0.01$^{a\#}$	1.15±0.03$^{a\#}$
Week 0	1.56±0.11^{a*}	2.33±0.08^{b*}	2.29±0.13^{b*}	2.31±0.22^{b*}	2.35±0.02^{b*}	2.29±0.09^{b*}
Week 10	1.15±0.02$^{a\#}$	1.53±0.04$^{b*\#}$	1.18±0.05$^{a\#}$	1.14±0.05$^{a\#}$	1.15±0.07$^{a\#}$	1.28±0.10$^{a\#}$

Values represent the mean±SEM (n = 7). Values for a given parameter in a row that do not share a common superscript are significantly different at $p<0.05$. In each vertical column with asterisk (*) mean differ significantly from Week -10 or hash (#) differ significantly from Week 0. NC: Normal control; PC: Positive control; T1: *T. indica* extract 5 mg kg^{-1}; T2: *T. indica* extract 25 mg kg^{-1}; T3: *T. indica* extract 50 mg kg^{-1}; Chi: Chitosan 300 mg kg^{-1}

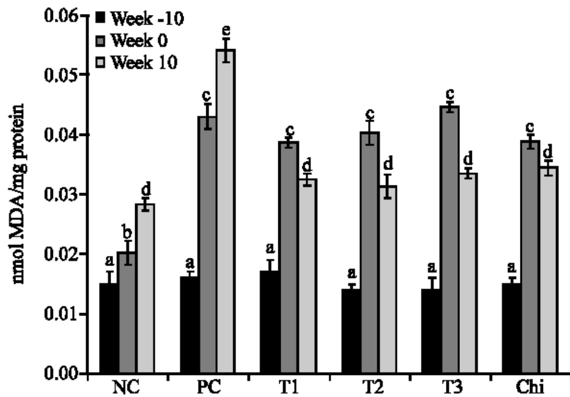

Fig. 1: Level of plasma malondialdehyde (MDA) of control groups and obese groups treated with *T. indica* extract and chitosan. Each bar represents the Mean±SEM (n = 7). Bars with a different superscript are significantly different at $p<0.05$ between groups on the respective week. All groups showed significant MDA levels different between weeks within groups. NC: Normal control; PC: Positive control; T1: *T. indica* extract 5 mg kg^{-1}; T2: *T. indica* extract 25 mg kg^{-1}; T3: *T. indica* extract 50 mg kg^{-1}; Chi: Chitosan 300 mg kg^{-1}

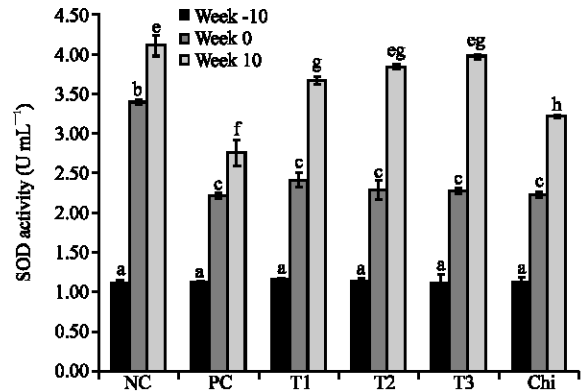

Fig. 2: Level of blood superoxide dismutase (SOD) activity of control groups and obese groups treated with *T. indica* extract and chitosan. Each bar represents the Mean±SEM (n = 7). Bars with a different superscript are significantly different at $p<0.05$ between groups on the respective week. All groups showed significant SOD levels different between weeks within groups. NC: Normal control; PC: Positive control; T1: *T. indica* extract 5 mg kg^{-1}; T2: *T. indica* extract 25 mg kg^{-1}; T3: *T. indica* extract 50 mg kg^{-1}; Chi: Chitosan 300 mg kg^{-1}

group, indicating that the supplementation of *T. indica* extract and chitosan could facilitate the hepatic HDL-C biosynthesis *in vivo* than the overall total cholesterol concentration.

Plasma malondialdehyde (MDA) level: The evaluation of lipid peroxidation products showed that the levels of MDA in the plasma were significantly increased in the groups fed a high-fat diet (Fig. 1). Subsequently, treatment of the groups with *T. indica* extracts and chitosan led to a significant decrease of MDA levels.

Antioxidant enzymes activities: The high-fat diet resulted in a significant decrease in the activities of SOD and GPx,

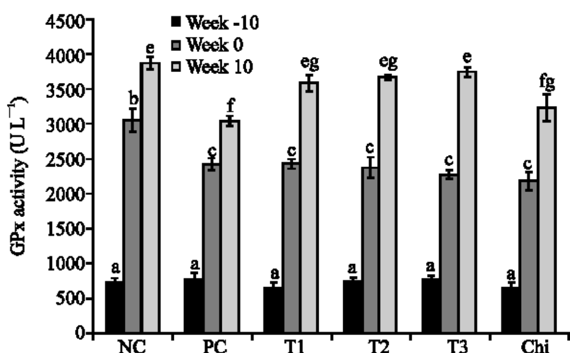

Fig. 3: Level of blood glutathione peroxidase (GPx) activity of control groups and obese groups treated with *T. indica* extract and chitosan. Each bar represents the Mean±SEM (n = 7). Bars with a different superscript are significantly different at $p<0.05$ between groups on the respective week. All groups showed significant GPx levels different between weeks within groups. NC: Normal control; PC: Positive control; T1: *T. indica* extract 5 mg kg^{-1}; T2: *T. indica* extract 25 mg kg^{-1}; T3: *T. indica* extract 50 mg kg^{-1}; Chi: Chitosan 300 mg kg^{-1}

when compared to the normal diet fed group (Fig. 2, 3). Remarkably, treatment of the obese-induced groups with *T. indica* extract and chitosan caused significant increment in SOD and GPx activities. Among all the treatments given, the groups treated with *T. indica* extract at 50 mg kg^{-1} showed the highest GPx level increment followed by *T. indica* extract at 25 mg kg^{-1}, Chitosan and *T. indica* extract at 5 mg kg^{-1} with about 64, 54, 48 and 47% increments, respectively. The increment of SOD activity were of highest for the group treated with *T. indica* extract at 50 mg kg^{-1} followed by *T. indica* extract at 25 mg kg^{-1}, *T. indica* extract at 5 mg kg^{-1} and chitosan with about 75, 67, 52 and 44% increments, respectively.

DISCUSSION

Obesity induction via dietary means in animal models has been considered as the most popular preference among researchers due to its high similarity of mimicking the usual way of obesity occurrence in human. It is generally known that high-fat diet is one of the major factors causing obesity and that long term intake of high-fat diet evokes significant increase in abdominal fat weight in mammals (Iwashita *et al*., 2002). After received their respective diet for 10 weeks, the high-fat diet rats gained significantly higher body weight than the normal rats, thus, verifying the obese status. This method of obesity determination is well practiced in a wide range of obesity research (Neyrinck *et al*., 2009). Though there was a significant difference in body weight between the high-fat and normal diet groups, no significant difference in daily food intake between groups was observed. This shows that differences of the diet content do not affect to the amount of food consumed by the animals. The high-fat diet groups continuously consumed similar quantities of food regardless to the higher calories content in the diet. As a result, the caloric intake was significantly higher in high-fat diet group than the normal group counterpart. The unchanged food intake and total caloric expenditure despite the higher caloric content in the diet is proportional to the increment of the body weight, hence, resulted in obese state. Supplementation of obese rats with *T. indica* extract at 25 and 50 mg kg^{-1} conversely causes a remarkable reduction of body weights compared to the positive control group and insignificantly different with that of the normal control group. The findings demonstrated that *T. indica* extract at 25 and 50 mg kg^{-1} are capable to prevent body weight gain concomitantly helping in maintaining the current body weight. Similar results had been reported by Shivshankar and Devi (2004) whereby mice supplemented with 10% tamarind pulp aqueous extract showed a significant reduction in their body weight after 4 weeks. Similarities of these findings strengthen the postulation that tamarind possesses weight reducing properties. It has been suggested that the high digestible starch and acidic content of tamarind extract cause alterations in the colonic mucosa and affected the microfloral content of the colon thereby aggravating the adverse effects of fatty diet and obese condition (Shivshankar and Devi, 2004).

It is well established that elevated blood lipids levels constitute the major risk for cardiovascular disease including atherosclerosis (Goldenberg and Glueck, 2009). In addition, hyperlipemia is also induced by the secondary effect of diabetes (Tan *et al*., 2005). In the present studies, apart from weight reducing ability, it was observed that *T. indica* extract supplementation was found to significantly decreased the levels of total cholesterol, LDL-C and triglyceride and increased more than 50% of HDL-C level in plasma of the obese group treated with *T. indica* extract, which reversed the effect of high-fat diet consumption alone. The increased HDL-C levels were found to be in a dose-dependent manner. Interestingly, the groups treated with *T. indica* extract at 50 mg kg^{-1} showed the highest HDL-C level increment (73%) followed by 25 mg kg^{-1} (72%), 5 mg kg^{-1} (62%) and Chitosan (53%). Similar results obtained by Martinello *et al.* (2006) whereby supplementation of *T. indica* pulp organic extract to hypercholesterolemic

hamsters led to an increase levels of serum HDL-C (61%) and decreased of total cholesterol (50%), LDL-C (73%) and triglyceride (60%) levels. In our experiment, a crude extract from *T. indica* was used to treat obese animals. Therefore, a limitation of this study is the inability to determine the specific components of the extract that mediate the effects observed. However, the reduction of plasma triglycerides and cholesterol levels is believed to be associated with the epicatechins contents in *T. indica* (Luengthanaphol *et al*., 2004; Chan *et al*., 1999). Crespy and Williamson (2004) suggested that long-term feeding of epicatechins could be beneficial for the suppression of high-fat diet-induced obesity by modulating lipid metabolism and could also reduce the risk of coronary disease.

Additionally, *T. indica* pulp extract was observed to have strong antioxidant effect in hyperlipidemia and prevents lipid peroxidation. In present study, supplementation with *T. indica* pulp extract in obese induced animals reduce lipid peroxidation indicated with low MDA concentration as much as 16, 22 and 25%, respectively when treated with 5, 25 and 50 mg kg^{-1} extracts in contrast to the high fat diet group without the treatment. The reduced MDA concentration despite to high oxidative stress from fat concentrated diet suggesting a protective effect of tamarind against oxygen free radicals in the biological system. In contrast, the increased level of oxidative stress found in the positive group clearly demonstrated that high fat consumption attributes to increased oxidative stress. Taken together, these results underline the antioxidant properties of *T. indica* extracts in preventing lipid peroxidation as similarly reported by Martinello *et al.* (2006). *Tamarindus indica* pulp extract has been demonstrated to have high polyphenols and antioxidant activity *in vitro* (Khairunnuur *et al*., 2009). Apart from antioxidative effect, tamarind supplementation was found to improve antioxidant defense system by enhancing the antioxidant enzymes activities *in vivo*. The groups treated with *T. indica* extracts showed a significant elevation in their SOD and GPx activities compared to the untreated rats. These results are aligned with the reduction of MDA levels as discussed earlier. The ability of *T. indica* extracts to suppress lipid peroxidation could be due to the anti-free radical activities of its phenolic components, known to act as free radical scavengers and/or to the increase in the activity of antioxidant enzymes regardless to the available lipid substrates. Considering the endogenous stress-related markers (SOD and GPx), these results suggest that *T. indica* extracts could potentially improve efficiency to the superoxide radical's conversion to hydrogen peroxide and increase SOD activity following deactivation of hydrogen peroxide by GPx. In conclusion, the present investigation proved that *T. indica* pulp extracts have lipid lowering and antioxidative effects in obese-induced rats apart from its weight reducing ability. In addition, the extract improves endogenous antioxidant biosynthesis by observing increase antioxidant activity of SOD and GPx. Due to the promising effect of tamarind extract in diet-induced obesity, further studies are required to isolate the active principle from this plant followed by identifying the probable mechanism of action in reducing plasma lipid and weight maintenance properties.

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