Antioxidant and cardio protective effect of palm oil leaves extract (standardized ethanolic fraction) in rats' model of saturated fats induced metabolic disorders

Zaid O Ibraheem[1,2], Munnavar Satar[2], Nor A. Abdullah[4], Hassaan Rathore[2], Young Chia Tan[2], Faiz Uldin[2], Rusliza Basri[1], Mohammad H Abdullah[2] and Edward John[3]

[1]Department of pharmacology, Faculty of Medicine and Health Sciences, University Putra Malaysia, Selangor, Malaysia
[2]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, 11800 Penang, Malaysia [3] Department of physiology, University College Cork, Cork, Ireland [4] Department of pharmacology, faculty of medicine, Universiti Malaya, Kuala Lumpur, Malaysia

Abstract: Recently, it is suggested to use POLE (palm oil leaf extract) as a nutraceutical health product in food industry due to its newly discovered content of polyphenols and antioxidant vitamins. In the experiment, the antioxidant and anti-lipid-peroxidation activities of the extract were confirmed using; DPPH (1-diphenyl-2-picryl-hydrazil) radical scavenging activity, ferric ion induced lipid peroxidation inhibition, reducing power and hydrogen peroxide scavenging activity assays. The cardio-protective activity was studied in vivo using a model of metabolic syndrome induced by high fat diet. Lipid profile, obesity indices, renal tubular handling of water and electrolytes, blood pressure and arterial stiffness were measured at the end of the treatment period. Sprague Dawley rats weighing 150-200 g were divided into six groups, viz; group C; was treated as a negative control and fed with standard rodents chow, group H; was treated as a positive control and fed with an experimental diet enriched with saturated free fatty acids for 8 weeks, groups HP0.5, HP1 and HP2 which were fed with 0.5,1 and 2 g/kg (body weight) /day of POLE orally during the last 24 days of the high fat diet feeding period and group P; fed with highest dose of POLE. Results revealed that POLE possesses a cardio-protective effect which is ascribed to its content of polyphenols.

Keywords: Cardio-protective, polyphenols, arterial stiffness, lipid profile and blood pressure.

INTRODUCTION

Botanical nutritional sources represent a useful protective supplement against the cardiovascular complications of metabolic disorders (Weaver *et al.,* 2008). Metabolic syndrome is widely disseminated that it is found in approximately 20% to 30% of a middle-aged people in the highly industrialized countries (Hansen 1999). For example, in the United States, it is present in about 25 to 50% of the population (Keller 2003). Metabolic syndrome is characterized by visceral obesity, hyperglycemia, glucose intolerance, hyperlipidemia and hypertension Gopala *et al.,* 2010). These abnormalities result in higher oxidative stress, pro-thrombotic and pro-inflammatory activities and higher arterial stiffness (Frisbee 2006).

Food rich in saturated fats has a negative impact on metabolic status and integrity of cardiovascular system. Long term ingestion of SAFFAs raises the blood pressure and deteriorates endothelial function through several interconnected mechanisms (Stampfer *et al.,* 1999 and Duvallw 2005).

Planning a healthy diet rich in antioxidant phytochemicals is one of the ways to avoid progression of metabolic and cardiovascular abnormalities. Nowadays, palm oil leaf extract is being used as a health product in food industry. The study investigated its potency as an antioxidant rich product to limit progression of metabolic syndrome related disorders (Mielke 1996).

Oil palm (*Elaeis guineensis*) is cultivated mainly in tropics. It represents the second largest contributor to vegetable oil consumption after soybean oil. Its fronds are the major waste products of palm oil industry (Mielke 1996). Nowadays, claims rose for using their extract (palm oil leaf extract (POLE)) in food industry as a beneficial health product especially after it has been discovered that it contains polyphenols (mainly glycosylated flavnoids and catechin), carotenoids and tocopherols (Irine-Runniea *et al.,* 2003). These compounds possess a chain breaking antioxidant activity. They interfere with generation of reactive oxygen species (ROS) which are the ubiquitous derivatives of oxygen metabolism in the biological system. ROS play an important role in the patho-physiology of cardiovascular dysfunctions associated with metabolic disorders (Agostino *et al.,* 2003). A previous study revealed that POLE, as an antioxidants rich product, reduces low density lipoprotein production (LDL) (Salleh *et al.,* 2002).

The study aims to evaluate the antioxidant and the possible cardio-protective activity of POLE using high fat diet model for cardiovascular abnormality induction.

**Corresponding author:* e-mail: z_79ph@yahoo.com

MATERIAL AND METHODS

Experimental animals

Male Sprague-Dawley rats weighing 150-200 g, obtained from the animal house facility of the School of Pharmaceutical Science/Universiti Sains Malaysia, were used in the study. The animals were acclimatized for one week before commencing the experiments. Animals were divided into 6 groups (6-8 animals in each group), viz ; C; fed with standard rodents chow for 8 weeks, H; fed with an experimental SAFFAs rich diet for the same period, HP0.5, HP1 and HP2; were fed with 0.5, 1 and 2 g/Kg (body weight)/day of POLE during the last 24 days of the high fat diet feeding period (from day 34 to day 58) and P2; fed with 2 g/Kg (body weight)/day POLE along with standard chow for 24 days. The experiments were approved by the Animals Ethic Committee, Universiti Sains, Malaysia.

Experimental high fat diet

An experimental high fat diet was prepared to be iso-nitrogenous. The quantity of all the essential nutrients was kept constant as percent of energy except for fats and carbohydrates (table 1). The diet was stored in the fridge and given daily after discarding food pellets of the previous day.

Table 1: Composition of the experimental diet as compared to the standard chow diet as a percent of total calories

Constituents	Standard chow diet		Experimental diet	
	Wt.(g)	Calories	Wt (g)	Calories
Protein	0.20	0.8	0.20	0.8
Carbohydrates	0.77	2.38	0.20	0.8
Fat	0.03	012	0.18	1.447
Cholesterol	-	-	0.01	-
Cholic acid	-	-	0.0025	-
Calcium	0.012	-	0.012	-
Phosphate	0.012	-	0.012	-
Sodium	0.021	-	0.021	-
Potassium	0.007	-	0.007	-
Total	1	3.3	0.074	3.3

The extract

Ethanolic fraction of POLE was obtained from (**N**ova Laboratories Sdn. Bhd. (179832-D). This fraction was chosen as most of polyphenols concentrate in it. Its phenol content was assessed using Folin Ciocalteu test. HPLC analysis was performed to detect the type of the polyphenols present in the extract. The in vitro antioxidant activity was assessed using free radical scavenging activity (FRSA), hydrogen peroxide scavenging activity, reducing power assay and anti lipid peroxidation assays. The extract was prepared by chopping and freeze drying of the leaves for 24 hours. After that the dried leaves were pulverized and the extract was prepared by soaking the powder with absolute alcohol 1:20 (w/v) for two days. Then it was filtered and the residue was re-extracted twice. After that, it was dried till the solvent is completely removed.

In vitro assessment of antioxidant activity

Total phenol content

Total phenolic content was determined using method described by Singleton and Rossi with some modifications (Singleton *et al* 1965). Polyphenols react with Folin Ciocalteu reagent in the presence of sodium carbonate to yield a colored complex whose maximum absorbance is 725 nm (Analytikijena 200-2004 spectrophotometer). Gallic acid (0.01-0.4 mM) was used to make the standard curve (0.01-0.4 mM). The results were expressed as mg of Gallic acid equivalents (GAEs)/g of extract.

Free radical scavenging activity

The free radical scavenging activity of the ethanolic fraction of POLE was done by measuring the ability of the extract to quench 1, 1-diphenyl-2-picryl-hydrazil (DPPHº) free radical using method of Shimada *et al* (1992). Eight times serial dilution was done for a solution of 1mg/ml of the extract then 3/ml of each dilution was mixed with 1ml of 0.1M DPPHº dissolved in methanol. After half an hour, the absorbance was measured at 517 nm. Then EC_{50} (Extract concentration of that inhibits 50% of the original DPPHº activity) was calculated from the plot of the absorbance versus the concentration. EC_{50} for the extract was compared to that of vitamin C and butylated hydroxytoluene (BHT) (Hristea *et al.*, 2002).

Reducing power assay

Reducing power of the extract was measured according to method of Oyaizu (1986). Different concentrations of the extract prepared by serial dilution of 1 mg/ml aqueous solution of the extract to prepare concentrations ranging from 8 µg/ml to 1 mg/ml. Each one of these concentrations was mixed with a mixture of 2.5/ml of 0.2M phosphate buffer (pH=6.6) and 2.5/ml of aqueous solution of 1% potassium ferocyanide [K3Fe(CN)6]. Then the mixture was incubated at 50°C for 20 min. and 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture. The mixture was then centrifuged for 10 min. at 3000 RPM. The supernatant was mixed with 2.5/ml distilled water and ferric chloride ($FeCl_3$). Finally, the absorbance was measured at 700 nm. The absorbance of the reaction mixture with different concentrations of the extract was compared with control which was prepared by adding 1 ml of distilled water instead of the extract. Percent of absorbance propagation was calculated for each concentration. The concentration of the extract

versus absorbance or the percent of absorption increase was plotted and compared with that of different concentrations of Vitamin C and butylated hydroxytoluene (BHT) (Gulcin *et al.,* 2005).

Hydrogen peroxide scavenging activity

It is determined by method of Oktay *et al* (2003). A solution of 2/mM hydrogen peroxide (H2O2) was prepared in 0.1/mM phosphate buffer saline (pH=7.4). Different concentrations of the extract prepared by serial dilution of a 1mg/ml in 70% ethanol to have a series of concentrations ranging from 8 µg/ml to 1 mg/ml. Mixtures of each extract concentration and hydrogen peroxide solution were incubated for 10 min. and the absorbance was determined using a UV-spectrophotometer at a wavelength 230 nm. Absorbance of each concentration was compared to that of control which was prepared by adding 70% ethanol instead of the extract. Percent of hydrogen peroxide scavenging ability was measured and a plot of this percent versus each concentration was plotted. The relationship was compared to that of some standard antioxidants, such as; vitamin C and butylated hydroxytoluene (BHT) (Zhenbao *et al.,* 2007).

Anti-lipid peroxidation assay

Anti-lipid peroxidation capacity was measured according to Kimuya, *et al.,* (1981). The test relies on the phenomenon of malonyldialdehyde (MDA) release as decomposition end products after exposure of cell membrane to oxidative stress. The release of malonyldialdehyde could be detected spectrophotometrically through its ability to form a complex with thiobarbituric acid (TBA) and form a MDA-TBA adduct. This complex is a pink colored complex with λmax 532 nm. In this test, a rat liver homogenate was mixed with iron and different concentrations of the extract were used to induce lipid peroxidation and MDA release. The amount of MDA released with each concentration was compared to that of control and the lipid peroxidation inhibition curve was compared with that of vitamin C. The rat liver was homogenized after being removed by abdominal dissection and perfused with cold HBSS (Hangs Balance Salt Solution) using peristaltic pump (Watson Marlow 323). HBSS is a calcium free electrolytic solution made up of 136.9/mM sodium chloride, 5.37/mM potassium chloride, dibasic sodium phosphate 0.34/mM, Potassium Phosphate 0.44/mM and D-glucose 5.55/mM. Homogenization was done using ice cold 0.15M potassium chloride and a tissue homogenizer at cold temperature. The homogenate was centrifuged at 3000RPM for 15 minutes at 4^{o}C. The supernatant was taken to study the *in vitro* lipid peroxidation. After that, 100µl of different concentrations of the extract ranging from 8µg/ml -1mg/ml were mixed with 500µl of the supernatant and 1ml of 0.15M KCl solution. Then 0.2mM ferric chloride FeCl3 solution was added. The mixture was incubated at 37^{o}C for 30 min. to induce lipid peroxidation. The reaction was stopped by adding TBA-TCA-HCl-BHT solution. TBA-TCA-HCl-BHT solution is made up of 1.68g TCA (trichloroacetic acid), 41.6g TBA (thiobarbituric acid), 10/ml of 125M HCl and 1/ml of 1.5g/ml of ethanolic solution of BHT (butylated hydroxytoluene). BHT stops the cascade of sequential reactions of lipid peroxidation while the rest constituents facilitate formation of the colored TBA-MDA adduct that can be detected spectrophotometrically. Then the reaction mixture was heated for 60 minutes at 90^{o}C, cooled at room temperature and centrifuged at 5000 RPM for 15 min. After that, the absorbance of each mixture was measured at 532nm using a visible light spectrophotometer. The control was prepared by adding 70% ethanol instead of the extract and absorbance was used to calculate the percent of lipid peroxidation inhibition. Percent of inhibition for each concentration was plotted against each concentration and the plot was compared to that of vitamin C. Sample blanks were prepared by the same procedure without adding ferric chloride (Yam *et al.,* 2007).

Biochemistry study

Cholesterol and triglyceride were measured on days 50 and 58 for all the treated groups using auto-analyzer (Cherwell biochemistry analyzer, Spain). 24 hour urine samples were obtained during the mentioned days and urinary sodium concentration was obtained using flame photometer (Jenway PFP7). Urine flow rate and absolute excretion of sodium were calculated using the standard equations. Oral glucose tolerance test was done at the end of the treatment period before commencing the acute study and after 12 hours fasting. Glucose was given in a dose of 5 gm/kg (B.W) orally using oral gavage. Serum glucose in mmol/l was measured using glucose meter (ACCU-CHEK® advantage blood glucose monitoring system Roche Diagnostics Corporation, Indianapolis, USA) at 0, 30, 60, 120 and 180 min after glucose administration. Then glucose tolerance curve and area under the curve (AUC) were determined for each group (Islam *et al.,* 2009).

Acute study

After one day of performing the OGTT, rats were fasted for overnight and underwent a surgical sessions; in which both abdominal and neck incision were done, after eight hours fasting under sodium pentobarbital (Nembutal®, CEVA, France) anesthesia. Right jugular vein and both left carotid and left iliac arteries were cannulated with polyethylene tubing (PE50, Portex limited Hythe, Kent, England) for fluid administration and measuring the hemodynamic and arterial stiffness parameters respectively. The carotid artery catheter was pushed into the level of the aortic arch while the iliac one was pushed up to the iliac bifurcation. Both arterial cannulas were connected to pressure transducers (P23 ID Gould,

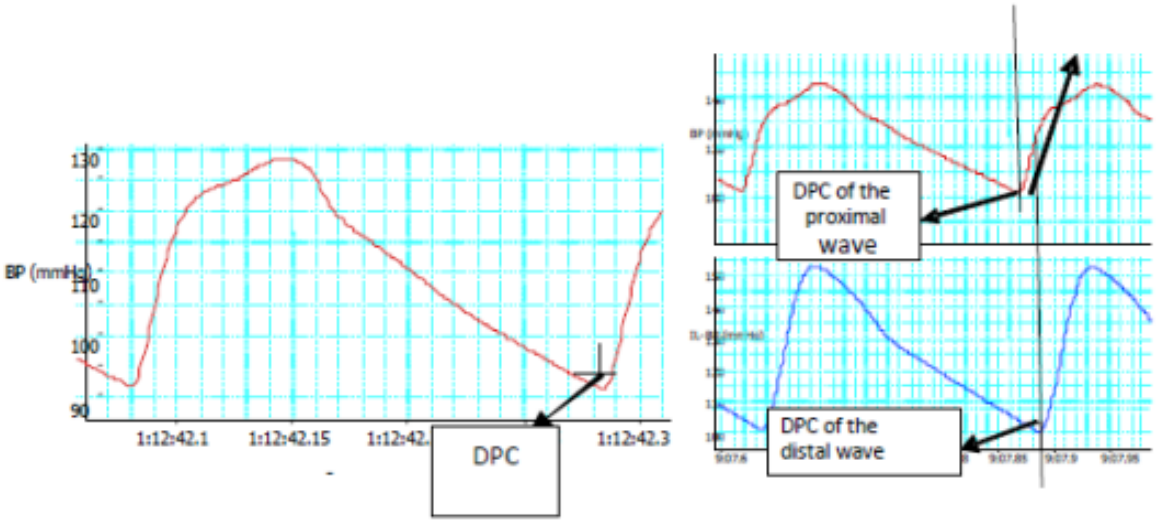

Fig. 1: (a) Measurement of the diastolic phase center (DPC) for a wave. DPC is the average point between the two points that lay 1 mmHg above the deepest points in both the upstroke and down-stroke limbs of the pulse wave (b) Measurement of the pulse wave velocity from both the carotid and iliac arteries wave fronts.

Statham Instrument, Nottingham, UK) linked to a computerized data acquisition system (PowerLab®, AD Instrument, Sydney Australia). Pulse waves were detected at a sampling rate of 400 Hz. At the end, the rats were euthanized with the lethal dose of pentobarbital sodium (200 mg/kg) and the full contour of aorta was exposed. The distance between the tips of the two cannulas (the pulse wave propagation distance) was measured through placing a damp silk thread over the contour of the artery and marking the tips. Then the fat depot was collected to determine the obesity indices and both kidneys were collected to determine kidney index and to perform biochemical studies on renal homogenate and histological studies. At the end, diastolic arterial pressure (DAP), systolic arterial pressure (SAP), mean arterial pressure (MAP), pulse pressure (PP) were obtained from the proximal pressure wave form displayed in the chart software (PowerLab®, AD Instruments, Sydney, Australia) (fig. 1). Pulse wave velocity (PWV) was calculated through computing both the propagation time and propagation distance. The propagation time was calculated through analyzing both the proximal and distal wave fronts through diastolic phase center method (DPC) as described in (fig. 1) (Richard *et al.,* 2001).

STATISTICAL ANALYSIS

Results were expressed as mean ± s.e.m. One way ANOVA followed by Tukey test was used for statistical analysis at 95% confidence level using students pack SPSS program version 16.

RESULTS

Extract

Phytochemical analyses revealed presence of polyphenols in a concentration of 52.4/mg GAE (Gallic acid equivalents) /gm of the pure powdered extract. The HPLC analysis showed that catechin and ferrulic acid were the main existing polyphenols. They present in a concentration of 0.55% and 0.63% of the pure powdered extract respectively. The in vitro antioxidant potential of extract was confirmed in vitro as seen (table 2).

Table 2: Results of in vitro tests of the antioxidant power assessment.

Assay	POLE	VitC	BHT
Total phenol content (mg GAE/ gm of pure extract)	52.4	-	-
DPPH Ec_{50} µgm/ml	35	18	11
H_2O_2 reducing power assay Ec_{50} µgm/ml	72	40	32
Anti-lipid peroxidation increase Ec_{50} µgm/ml	140	28	-
Reducing power assay IC_{50} µgm/ml	41	4	2

Metabolic syndrome assessment study

High fat diet treatment for two months raised lipid profile significantly as compared to control ($P<0.05$) (table 3). This increase is accompanied by a significant increase in blood pressure and arterial stiffness ($P<0.05$) (table 3). Moreover, it significantly raised obesity index and lipid profile ($P<0.05$) with a noticeable upward shifting of the

glucose tolerance curve and increase in values of areas under the curve (AUC) (fig. 2).

Co-administration of POLE as a fibers and antioxidant polyphenols rich diet is suggested to prevent all consequences of metabolic errors caused by Saturated fats ingestion. Results of obesity index and glucose tolerance assessment for the groups given POLE along with the experimental high fat diet did not show any obvious decrease while lipid profile parameters were statistically significantly decreased in comparison to control.

Renal tubular function

Results of tubular function study showed that high fat diet stimulates tubular re-absorption; results of urine flow rate and absolute excretion of sodium was significantly lower as compared to control (P<0.05) (table 3). Ingestion of POLE along with the high fat diet during the last 24 days of the feeding period has diminished the over activity of the renal tubular system as depicted in results of tubular function study (table 3).

Table 3: Results of metabolic study for renal function and metabolic syndrome assessment during days 50 and 58 of the *ad libitum* a high fat diet feeding period. Results are expressed in mean±S.E.M.* Indicates statistical significance as compared to control (P<0.05). # indicates statistical significance as compared to HFD group (P< 0.05). CHO=cholesterol, T.G=triglycerides, URF= Urine flow rate and Abs. Na^+ = Absolute excretion of sodium.

Group		UFR (µl/min/ 100 gm B.W)	Abs. Na^+ Excretion (mmol/hr.)	FE Na^+	CHO (mmol /L)	T.G (mmo l/L)
C	d50	2.18± 0.20	0.0162 ±0.002	0.49± 0.027	1.14± 0.017	0.42± 0.008
	d58	2.06± 0.13	0.0178 ±0.002	0.49± 0.035	1.19± 0.016#	0.41± 0.009#
H	d50	1.42± 0.10	0.010± 8E-04	0.32± 0.025	1.35± 0.028	0.54± 0.012
	d58	1.35± 0.08	0.010± 9E-04	0.39± 0.021	1.46± 0.033*	0.61± 0.008*
HP0.5	d50	1.31± 0.06	0.008± 0.001	0.34± 0.064	1.49± 0.092	0.66± 0.063*
	d58	1.19± 0.08	0.008± 6E-04	0.38± 0.043	1.65± 0.129*	0.67± 0.040*
HP1	d50	1.39± 0.10	0.011± 0.001	0.46± 0.047	1.37± 0.075	0.51± 0.047
	d58	1.36± 0.12	0.0124± 0.001	0.55± 0.043	1.43± 0.056	0.54± 0.036
HP2	d50	1.42± 0.11	0.0110± 8E-04	0.45± 0.045	1.35± 0.099	0.52± 0.055
	d58	1.71± 0.10	0.0144± 7E-04	0.38± 0.033	1.33± 0.099	0.51± 0.061
P2	d50	2.72± 0.25	0.0162± 0.002	0.56± 0.048	1.08± 0.013	0.43± 0.009
	d58	2.6± 0.24$	0.0178± 0.002	0.56 ±0.06	1.04± 0.012	0.40± 0.007

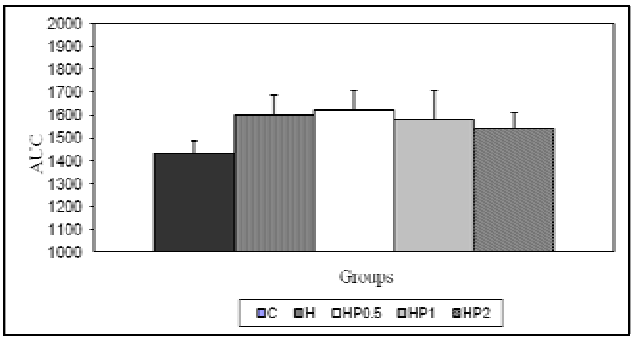

Fig. 2: Results of the area under the curve (AUC) in (mol $L^{-1}hr.^{-1}$) of the glucose tolerance curve for the treated groups at the end of the treatment period.

Cardiovascular parameters

The deleterious effect of SAFFAs on cardiovascular system was obvious from results of the acute study. There was a statistically significant increase in blood pressure (P<0.05) (table 4). This was accompanied by a statistic-cally significant increase in arterial stiffness as depicted in results of pulse wave velocity (P<0.05) (table 4).

Table 4: Acute study parameters at the end of the treatment period * Indicates statistical significance as compared to control (P<0.05). Number indicates statistical significance as compared to HFD group (P<0.05). MAP=mean arterial pressure and PWV=pulse wave velocity.

Group	MAP (mmHg)	PWV(m/s)
C	109±2.4#	4.73±0.072#
H	128±3.5*	5.62±0.243*
HP0.5	124±3.2	5.36±0.3
HP1	121±6.6	5.2±0.3
HP2	110±5.1	4.83±0.2
P2	102±4.1	5.18±0.3

DISCUSSION

The antioxidant activity of POLE was confirmed by the in vitro tests which depicted the power of the extract to scavenge and reduce the released free radicals. Free radicals are released during metabolic processes. They have a powerful predilection to snatch electrons from cell membrane lipids which are endowed with a great deal of unsaturated free fatty acids. This results in changing these fatty acids into fatty acid radicals. Fatty acid radicals tend to attack the surrounding environment to produce a chain propagation reaction of free radicals generation and lipid peroxidation. This results in damaging the membranous structures leading to cellular degeneration and necrosis (Akhgari *et al.,* 2003). The ability of POLE to counteract these processes was screened through its ability to inactivate the DPPH radical turning it into an inactive neutral one. The extract could have limited the existence

of hydrogen peroxide by donating an electron which converts hydrogen peroxide to water and oxygen. The ability of the extract to act as a reducing agent was tested by the reducing power assay which tested the power of the extract to counteract progression of Prusian Blue reaction. Ferric ion is one of the inducers of lipid peroxidation. It has a tendency to be reduced to ferrous ion inducing a famous reaction known as Fenton reaction. In antilipid peroxidation assay, lipid peroxidation is triggered through incubating ferric ion with liver homogenate which is endowed with a great deal of membranous unsaturated free fatty acids. Malonyldialdehyde release was used as a lipid peroxidetion marker. The antioxidant power of the extract halted progression of lipid peroxidation and suppressed malonyldialdehyde release. This potency can be an indicator for the polyphenol content and the potency of these polyphenols to antagonize Fenton reaction and the progression of lipid peroxidation (Narayanan *et al.,* 2009).

The phytochemical and HPLC analyses revealed presence of polyphenols. Catechine and ferrulic acid were constituting the majority of them. Catechin is a tricyclic flavonoid derivative. It possesses an antioxidant activity due to the presence of phenolic groups attached to benzene ring (Chumbalov 1995). Ferrulic acid is a phenylpropanoid derivative, found in plant cell wall as a covalent side chain attached to arabinoxylan and cellulose of the cell wall. Inside the plant, it serves to crosslink lignine to polysaccharides adding some rigidity to the cell wall. Previous studies reveal that after ingestion, ferrulic acid is absorbed in gastrointestinal tract after cleavage of its linkage with lignines by pancreatic acids (Pan *et al.,* 1999). It has better bioavailability in plasma as compared to other polyphenols (Scheliner 1968). Ferrulic acid has a unique antioxidant power due to the presence of carboxyl group in its structure which acts as a free fatty acid anchor in membranous structures resulting in higher anti lipid peroxidation effect (Kanski *et al.,* 2002).

Ingestion of high amount of SAFFAs has a negative impact on the metabolic status. Our results revealed that metabolic changes had occurred in the group fed with the saturated fats. These changes were chractarized by hypertriglycreridemia and visceral obesity. The oral glucose tolerance curve was not affected. These changes are attributed to SAFFAs uptake induced hyperinsulinemia (Manco *et al.,* 2000). which may occur due to the direct effect of SAFFAs on β-cells of Islets of Langerhans and through increasing the release of some gastrointestinal peptides which have a stimulatory effect on insulin secretion after high amount of SAFFAs ingestion (Rutter 2003). Hyperinsulinemia down-regulates insulin receptors and suppresses cellular uptake of both glucose and potassium (Morgan 2010). SAFFAs induce insulin resistance in muscles and hepatocytes directly by increasing the expression of PKC-O (Protein-Kinase-C-theta). PKC-O phosphorylates IRS-1 (Insulin Receptor Substrate-1) and this reduces the ability of IRS-1 to phosphorylate tyrosine that is required for phosphotidylinositol phosphorylation. Phosphotidylinositol phosphorylation is crucial for glycogen building and glucose uptake (Kragen *et al.,* 1991). Engorgement of visceral fats with high amount of lipids trigger the inflammatory pathway in adipocytes (Kragen *et al.,* 1991). The adipose tissue does not act merely as an energy storage sites, but it acts as an endocrine gland which secretes factors related to glucose homeostasis, food uptake, energy metabolism and immune function (Lago *et al.,* 2007). Overloading of the adipocytes with triglycerides leads to their hyperatrophy and dysfunction, and makes them release their content of adipokines (Kennedy *et al.,* 2009). Macrophage chemotactant factor-1 (MCP-1) is one of the important adipokines released by adipose tissue. It induces macrophages recruitment there (Guilherme *et al.,* 2008). It was estimated that in lean subjects, 5-10% of the total macrophages are accumulated in the adipose tissue while 50% accumulate there in obese individuals (Weisberg *et al.,* 2003). The accumulated macrophages release inflammatory cytokines as; tumor necrotic factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL6). These agents reduce triglyceride deposition in adipocytes, stimulate lipolysis and make the adipocytes more resistant to insulin (Guilherme *et al.,* 2008 and Shihang *et al.,* 2006 and Haversen *et al.,* 2009). This triggers accumulation of more free fatty acids and triglycerides in blood and in muscles as yellow deposits. Deposition of fatty acids in muscles hampers the oxidative phosphorylation and insulin stimulated glucose transport (Guilherme *et al.,* 2008 and Weisberg *et al.,* 2003 and Faria *et al.,* 2002). Consumption of high SAFFAs increases the oxidative stress and adversely affects endocrine homeostasis resulting in poorly controlled glucose tolerance (Carmiel-haggai *et al.,* 2005). It was found that; ingestion of SAFFAs especially palmitate induces accumulation of ceramide and diacylglycerol as end products of SAFFAs metabolism. This suppresses insulin signaling and promotes insulin resistance (Chavez 2003). According to the portal theory, liver shows an exquisite sensitivity to the high fat diet (Arner 1998). that ingestion of isocaloric diet with an increased amount of fat up to 35-45% has a potent daunting effect on insulin sensitivity in liver (Kabir *et al.,* 2005). SAFFAs trigger hyperinsulinemia through impairing the first pass effect of insulin clearance (Kragen *et al.,* 1991). They directly induce insulin insensitivity through inhibiting IRS-1. Meanwhile, ingestion of a hypercaloric diet with high percent of fat induces insulin insensitivity in liver and peripheral tissues (Kabir *et al.,* 2005).

Co-administration of POLE as a fibers and antioxidant polyphenols rich diet is suggested to prevent all consequences of metabolic errors caused by Saturated fats

ingestion. Results of obesity index and glucose tolerance assessment did not show any obvious decrease while lipid profile parameters were decreased after high fat diet ingestion to be statistically insignificantly different in comparison to control.

Results of tubular function study showed that high fat diet stimulates tubular re-absorption; results of urine flow rate and absolute excretion of both sodium and potassium were significantly lower as compared to control ($P<0.05$) (table 3). This may be attributed to hyperinsulinemia which triggers the basolateral Na^+-K^+ ATPase pump in DCT (DeFronzo 1981 and Herlitz *et al.,* 1996). Moreover, SAFFAs trigger renin-angiotensin system and increase expression of Bummetonide sensitive Na^+-K^+-$2Cl^-$ co-transporters and the subunits of the apical membrane Na^+ channels in thick ascending limb of loop of Henle (Jian-Song *et al.,* 2004). Ingestion of POLE along with the high fat diet during the last 24 days of the feeding period has diminished the over activity of the renal tubular system as depicted in results of tubular function study (table 2). This amelioration may be attributed to the non antioxidant action of polyphenols as it was found that polyphenols inhibit intracellular secondary messengers leading to a decrease in tubular function (Ramamoorythy *et al.,* 1990).

The deleterious effect of SAFFAs on cardiovascular system was obvious from results of the acute study. There was a statistically significant increase in blood pressure ($P<0.05$) (table 4), which was graded clinically as mild hypertension. This was accompanied by a statistically significant increase in arterial stiffness as depicted in results of pulse wave velocity ($P<0.05$) (table 4). These changes are attributed to release of inflammatory cytokines as interleukin 1, interleukin 6 and tumor necrotic factor after saturated fats ingestion. These cytokines are culminated deterioration of endothelial function (Cortan 1990). Endothelium plays a pivotal role in regulation of blood pressure through releasing of balanced amount of vasorelaxant factors as nitric oxide and vasoconstrictors as endothelin (Granger 2006). SAFFAS change the endothelium function toward releasing more vasoconstrictors that produce hypertension (Cortan 1990). Moreover, they stimulate renal tubular re-absorption of water and electrolytes raising the pressure as mentioned earlier (Herlitz *et al.,* 1996 and Jian-Song *et al.,* 2004). Previous studies declared that insulin resistance potentiates the sympathetic outflow along with over activation of renin- angiotensin system which augment hypertension (Harte *et al.,* 2005). Some biochemical changes occur in the arteriolar wall, known as arterial remodeling. They are characterized by disturbance in its content of elastin and collagen in favors of more elastin formation (Brasselet *et al.,* 2005). Hypertension and hyperlipidemia are among the triggering factors for this remodeling (Chatzizissis *et al.,* 2007). POLE co-administration along with the high fat diet has reduced the blood pressure and the arterial stiffness accordingly. This suggests the cardio protective effect of the polyphenols content of the extract. This effect is closely related to the abovementioned ability of the extract to limit sodium and water retention along with its ability to limit glucose intolerance and hyperlipidemia (Irine-Runniea *et al.,* 2003). Moreover, ingestion of antioxidants improves the endothelial function which is required for the optimum cardiovascular dysfunction (Duvallw 2005).

REFERENCES

Agostino V, Emiliano D and Stefano T (2011). Oxidative stress and vascular damage in hypertension:role of angiotensine II. *Int J hypertens*.**12**(6): 455-461.

Akhgari M, Abdollahi M, Kebryaeezadeh A, Hossini R and Sabzevari O (2003). Biochemical evidence for free radical induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. *Hum. Exp. Toxicol.,* **22**(4): 205-211.

Arner P (1998). Not all fat is alike. *Lancet,* **351**(9112): 1301-1302.

Brasselet C, Durand E, Addad F, Zen AA, Smeets MB and Laurent-Maquin D *et al*. (2005). Collagen and elastin cross-linking: A mechanism of constrictive remodeling after arterial injury. *Am. J. Physiol. Heart Circ. Physiol*., **289**(5): H2228-H2233

Carmiel-haggai M, Cederbaum AI and Nieto N (2005). High-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *FASEB J*., **19**(1): 136-138.

Chatzizissis YS, Coskun AU, Jonas M, Edeman ER, Feldman CL and Stone PH (2007). Role of endothelial shear stress in the natural history of coronary atherosclerosis and Vascular Remodeling: Molecular, cellular and vascular behavior. *J. Am. Coll. Cardiol.,* **49**(25): 2379-2393.

Chavez JA (2003). Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes. *Arch. Biochem. Biophys*., **419**(2): 101-109.

Chumbalov TK (1995). Catechin 7-rhamnoside from *Spiraea hypericifolia*. *Chem. Nat. Comp.* **12**(2): 232-233.

Cortan RS and Pober JS (1990). Cytokine-endothelial interactions in inflammation, immunity, and vascular injury. *J Am Soc Nephrol.* **1**(3): 225-235.

DeFronzo RA (1981). The effect of insulin on renal sodium metabolism. *Diabetologia,* **21**(3): 165-171.

DuvallwL (2005). Endothelial dysfunction and antioxidants. *MT Sinai J. Med.* **72**(2): 71-80.

Faria AN, Ribero-Filho FF, Gouveta-Ferreira SR and Zanella MT (2002). Impact of visceral fat on blood pressure and insulin sensitivity in hypertensive obese women. *Obesity,* **10**: 1203-1206.

Frisbee JC (2006). Vascular function in the metabolic syndrome and the effects on skeletal muscle perfusion: lessons from the obese Zucker rat. *Essays Biochem.,* **42**(1): 145-161.

Gopala A, Sharma VK and Ganti SS (2010). Rosiglatizone a journey that never completed. *Schwiez Med Wochenscher.,* **108**(39): 1485-1495.

Granger JP (2006). An emerging role for inflammatory cytokines in hypertension. *Am. J. Physiol. Heart Circ. Physiol.,* **290**(3): H923-H924.

Guilherme A, Vibrasius JV, Puri V and Czech MP (2008). Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat. Rev. Mol. Cell Biol.,* **9**(5): 367-377.

Gulcin I, Bershvili D and Gepdiremen A (2005). Anti-radical and antioxidant activity of total anthocyanins from *Perilla pankinensis decne*. *J. Ethnopharmacol.,* **101**: 287-293.

Hansen BC (1999). The metabolic syndrome X. *Ann. N. Y. Acad.* **892**(1): 1-24.

Harte AM, Philip C, Rajkumar C, Simon K, Jonathan S and Stephen K (2005). Insulin-mediated upregulation of the renin angiotensin system in human subcutaneous adipocytes is reduced by rosiglitazone. *Circulation,* **111**(15): 1954-1961.

Haversen L, Danielsson KN, Fogelstrand L and Wiklund O (2009). Induction of pro-inflammatory cytokines by long-chain saturated fatty acids in human macrophages. *Atherosclerosis,* **202**(2): 382-393.

Herlitz H, Widgren B, Urbanavicius V, Attvall S and Persson B (1996). Stimulatory effect of insulin on tubular sodium reabsorption in normotensive subjects with a positive family history of hypertension. *Nephroy Dials Transplantation,* **11**(1): 47-54.

Hristea EN, Hillebrand M, Caproiu MT, Caldararu H and Constantinesca T (2002). Scavenging the hydroxyl radical by 2,2-diphenyl-1-picrylhydrazyl. *ARKIVOC*, **2**(2): 123-132.

Irine-Runniea NM, Radzali M, Azizah H, Hapizah Nd, Mahinda Y and Suhaila M (2003). Antioxidant and hypocholesterolemic effects of *Elaies guineensis* frond extract on hypercholesterolemic rabbits. *ASEAN Food J.,* **12**(3): 137-148.

Islam MA, Khan MR, Hossain MS, Alam AH, Ibne-Wahed MI and Amran MS (2009). Oral glucose tolerance test (OGTT) in normal control and glucose induced hyperglycemic rats with *Coccinia cordifolia* l. and *Catharanthus roseus* L. *Pak. J. Pharm. Sci.,* 2**2**(4): 402-404.

Jian-Song XH, Min-Shi, Mark AK and Carolyn AE (2004). Effects of dietary fat, NaCl and fructose on renal sodium and water transporter abundances and systemic blood pressure. *Am. J. Physiol. Renal Physiol.,* **287**: 1204-1212.

Kabir M., Catalano KJ, Ananthnarayan S, Kim SP, Van-Citters SG and Dea MK (2005). Molecular evidence supporting the portal theory: A causative link between visceral adiposity and hepatic insulin resistance. *Am. J. Physiol. Endocrino. Metab.,* **288**(2): 454-461.

Kanski J, Stoyanova A and Butterfield DA (2002). Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems *in vitro*: Structure-activity studies. *J. Nutr. Biochem,,* **13**(5): 273-281.

Ram Weiss, James Dzura, Tania S Burgert, William V, Tamborlane *et al*. (2004). Obesity and metabolic syndrome in children and adolescence. *N. Engl. J. Med.,* **350**: 2362-2374.

Kennedy A, Martinez K, Chuang C, Lapoint K and Mcintosh M (2009). Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: Mechanisms of action and implications. *Am. J. Nutr.,* **139**(1): 1-4.

Kragen, EW, Clark, PW, Jenkins AB, Daley EA, Chisholm DJ and Storlien L H (1991). Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes,* **40**: 1397-1403.

Lago F, Gomez-Reino J and Gualillo O (2007). Adipokines as emerging mediators of immune response and inflammation. *Nat. Clinl. Prace. Rheumatol.,* **3**(12): 716-724.

Manco M, Greco A, Carristo E, Gniuli D, Degaetano A and Gasbarrini G (2000). Insulin resistance directly correlates with increased saturated fatty acids in skeletal muscle triglycerides. *Metabolism,* **49**(2): 220-224.

Mielke T (1996). Outlook for oils and fats from 1996 onwards with special emphasis on oil palm and kernel oil. PAC Seminars of the Palm Oil Res. Inst. of Malaysia (PORIM).

Morgan DA (2010). Differential effects of insulin on sympathetic nerve activity in agouti obese mice. *J. of Hypert* **28**(9): 1913-1919.

Narayanan BL, Rajkumar Pradeep LA, Arulanandam A, Babu NS, Gayathri T and Raju A (2009). Antioxiadant and anti-inflammatory activity of synthesized 3 (substituted) chromen-2-one. *Int. J. Pharm. Pharmaceul. Sci.,* **3**(2): 474-478.

Pan Gx and Leary GJ (1999). Reactivity of ferulic acid and its derivatives toward hydrogen peroxide and peracetic acid. *J. Agricul. Food Chem.,* **47**(8): 3325-3331.

Ramamoorythy AJ, Chidambaranathan N and Devi P (2010). Physio-Phytochemical screening and Diuretic activity of leaves of Pavetta indica Linn. *Pharmaceut. Sci. Res.,* **2**(8): 506-512.

Richard M, Fitch R, Mark E, Sullivan and Yi-Xin W (2001). Nitric oxide synthase inhibition increases aortic stiffness measured by pulse wave velocity in rats. *Cardiovasc. Res.,* **51**(2): 351-358.

Rutter GA (2003). Insulin Secretion: Fatty acid signalling via serpentine receptors. *Curr. Biol.,* **13**: 403-405.

Salleh M, Runnie I, Roach PD, Mohamed S and Abeywardena MY (2002). Inhibition of low-density

lipoprotein oxidation and up-regulation of low-density lipoprotein receptor in HepG2 cells by tropical plant extracts. *J. Agric. Food Chem.,* **50**(13): 3693-3697.

Scheliner R (1968). The metabolism of drugs and other compounds by the intestinal microflora. *Acta. Pharmaco. Toxicol.,* **26**: 332-342.

Shihang K, Inouye MV, Tzameti and Yin H (2006). TLR4 links innate immunity and fatty acids induced insulin resistance. *J. Clin. Invest.,* **116**(11): 3015-3025.

Stampfer MJ, JoAnn E, Ascherio, Alberto, Colditz and Graham A (1999). Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. *Am. J. Clin. Nutr.,* **70**(6): 1001-1008.

Weaver CM, Barnes S, Wyss JM, Kim H, Morre D M and Morre DJ (2008). Botanicals for age-related diseases: From field to practice. *Am. J. Clin. Nutr.,* **87**: 493-497.

Weisberg SP, Mccann D, Desai M, Rosenbaum M, Leibel RL and Ferrante AW (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.,* **112**(12): 1796-1808.

Yam MF, Basri R, Mohd Z and Zhari I (2007). Antioxidant and hepatoprotective effects of *Orthosiphon stamineus* Benth. standardized extract. *Am J. of Chin. Med.,* **35**(1): 115-126.

Zhenbao J, Fei T, Ling G, Guanjun T and Xiaolin D (2007). Antioxidant properties of extracts from juemingzi (Cassia tora L.) evaluated *in vitro*. *LWT-Food Sci. Tech.,* **40**(6): 1072-1077.