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Attenuation of High-fat Diet Induced Body Weight Gain, Adiposity and Biochemical Anomalies after Chronic Administration of Ginger (*Zingiber officinale*) in Wistar Rats

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Abstract: The aim of the present study was to investigate the anti-obesity effects of the ginger in a rat model of high-fat diet induced obesity. In the preventive study, male Wistar rats were fed high-fat diet along with different doses of ginger (0.25-1 g/kg/day, p.o. o.d). for 8 weeks. In the ameliorative study obese rats were selected after 8 weeks feeding of high-fat diet and then treated with different doses of ginger (0.25-1 g/kg/day, p.o. o.d). for 8 weeks along with high-fat diet or normal chow diet. The effect of these treatments on changes in feed intake, body weight, Lee's index, adipose tissue weight, core body temperature (an index of thermogenesis) and serum biochemicals were analyzed. In preventive study ginger treatment not only decreased body weight gain, Lee's index, adipose tissue weight but also enhanced thermogenesis and improved glucose and lipid homeostasis. These beneficial effects were attenuated by propranolol indicating the role of beta-adrenoceptors. Ginger treatment also ameliorated established obesity and associated biochemical consequences and more so when high-fat diet was replaced with normal diet signifying the importance of dietary modification in successful obesity therapy. Ginger treatment was found devoid of any toxicity as assessed by liver enzyme levels and weights of vital organs. The results suggest that chronic administration of ginger can inhibit the development of obesity and associated metabolic consequences in high-fat diet induced obesity without significant adverse effect.

Key words: Obesity, high fat diet, thermogenesis, ginger, adipose tissue

INTRODUCTION

Obesity is a chronic, multifactorial and serious public health problem that has reached pandemic proportion worldwide (Nguyen and El-Serag, 2010). Obese patients have more extensive visceral adipose tissue, anomalies of blood-glucose homoeostasis, elevated plasma triglycerides (TG) and low levels of high-density lipoprotein (HDL) cholesterol that further contributes to the later appearance of cardiovascular syndromes (Despres *et al*., 2006; St-Pierre *et al*., 2007). Moreover, obese and overweight patients are at higher risk from coronary artery disease, hypertension, hyperlipidemia, diabetes mellitus, cancers, gall bladder disorders, cerebrovascular accidents, osteoarthritis, restrictive pulmonary disease and sleep apnoea (Hameed *et al*., 2002; Afridi *et al*., 2003; Rouhi *et al*., 2006; Ebrahimi-Mameghani *et al*., 2008; Afoakwah and Owusu, 2011). Unfortunately, medications currently available have very poor outcome on long-term weight loss and are associated with various adverse effects some of which are serious in nature (Ioannides-Demos *et al*., 2006). Due to the absence of safe and effective antiobesity treatment, use of natural dietary supplements for weight loss has risen dramatically but therapeutically majority of them remains scientifically evaluated (Sharpe *et al*., 2006).

The medicinal properties of ginger has been used since ancient times in Indian, Arabic and Chinese herbal traditions and continues to be in use even in Western culture (Awang, 1992; Tapsell *et al*., 2006). The major chemical constituents of ginger rhizome include essential volatile oils and non-volatile pungent compounds The volatile oil components mainly consist of various terpenoids while as non-volatile compounds include the gingerols, shogaols, paradols, zingerone and their derivatives (Ali *et al*., 2008). Various well reported pharmacological activities of ginger include antiemetic, antiulcer, hepatoprotective, anti-asthmatic, anti-inflammatory, analgesic, antiplatelet, hypotensive, hypolipidemic, antihyperglycaemic, immunomodulator, antitumorigenic and antimicrobial actions (Afzal *et al*., 2001; Rouhi *et al*., 2006; Ali *et al*., 2008; Nicoll and Henein, 2009). Recently, ginger has been promoted as antiobesity agent and many antiobesity preparations contain ginger as an integral constituent (Sivakumar and Sivakumar, 2004; York *et al*., 2007). In fact, ginger is the most common used ingredient in antiobesity preparations

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(Sharpe *et al*., 2006). However, there is paucity in the literature regarding the effectiveness of ginger in obesity. Although reduction in body weight gain with ginger extracts when given prophylactically were noticed in chemical- (Goyal and Kadnur, 2006) and in high-fat diet-induced obese mice (Han *et al*., 2005) and rats (Nammi *et al*., 2009) but a detailed study examining both the prophylactic and ameliorative effect of ginger on high fat diet induced weight gain, adiposity and associated pathologies in rats is lacking. Moreover, the mechanism of antiobesity action and the effect of dietary modification on antiobesity activity of ginger have not been studied. Therefore, the current study has been designed to investigate the prophylactic and ameliorative effect of chronic ginger treatment in dietary obesity and associated pathologies in male wistar rats along with mechanism of action.

MATERIALS AND METHODS

Drugs and chemicals: The entire study was conducted between January 2009 and October 2009. 6-gingerol (Sigma, USA) was dissolved in ethanol for plotting standard curve. Propranolol (Cipla LTD, India) was suspended in 0.5% carboxy methylcellulose (CMC) before oral administration. All other chemicals employed in the present study were of analar grade.

Preparation of lyophilized ginger juice powder: Fresh ginger rhizomes were purchased from local market and were identified and authenticated by the Department of Botany, Punjab University, Chandigarh. Voucher specimen (PH-2010/0133) was deposited in the herbarium of the institute. After washing thoroughly juice of ginger was prepared on a juicer (Philiphs, India) and completely lyophilized as described previously by Malik *et al*. (2011a) (yield 5% of fresh ginger juice). The Lyophilized Ginger Powder (LGP) so obtained was stored in dessicator in an airtight container at low temperature and suspended in 0.5% CMC before oral administration.

Phytochemical screening and standardization: Phytochemical screening of the MC was carried out employing standard procedures and tests (Trease and Evans, 1989). LGP was standardized with reference compound 6-gingerol as well as with various bioactive compounds.

For 6-gingerol 1 g of the LGP was mixed with 70 mL of ethanol (95%), vortexed (3 min) followed by ultrasonication (65°). After 1 h volume was made up to 100 mL with ethanol (95%), mixed well and filtered. To 1 mL of the filtrate, 0.5 mL of potassium ferricyanide was added and 5 min later volume made up to 50 mL with 0.1 mM HCl and allowed to stand for further 15 min for complete colour formation. The absorbance of the sample was read spectrophotometrically (UV 1700, Shimadzu) at 660 nm and the content of 6-gingerol was calculated using the standard curve (Nammi *et al*., 2009).

For other bioactive estimations one gram of LGP was weighed accurately and suspended in 100 mL of water. The mixture was vortexed (3 min) followed by ultrasonication (30 min) and then centrifuged at 1500×g (10 min) to get supernatant sample solution for total phenolic content, total flavonoid, total tannin and ascorbic acid content.

Total phenolic and total flavonoid content was determined according to the methods of Singleton and Rossi (1965) and Zou *et al*. (2004), respectively. Determination of total tannins was based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution (Ranganna, 1986). Ascorbic acid was determined according to the method of Klein and Perry (1982).

Acute toxicity study: The acute toxicity of GP was performed according to Litchfield and Wilcoxon (1949).

Experimental diet: Obesity was induced by HFD, prepared by mixing 33% powdered normal chow (Ashirwad Diets, Punjab, India), 33% Nestle milk powder, 7% sucrose and 27% tap water by weight (Wilding *et al*., 1992). This diet provides 68% energy as carbohydrate, 20% as protein and 12% as fat and is reported to produce reliable weight gain (obesity) in wistar rats (Brown *et al*., 2001) while as Normal Chow (NC) provides 65% of energy as carbohydrate, 20% as protein and 4% as fat.

Animal treatments: Male wistar rats of 7-8 weeks of age were procured from the animal facility of the Institute. The animals were housed in standard polypropylene cages (two rats/cage) and maintained under controlled room temperature (25±2°C) with 12:12 h light and dark cycle. The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India were followed and prior permission was sought from the institutional animal ethics committee for conducting the study.

Experiment 1: Rats were randomly divided into various groups on the basis of their body weight. Group 1 and 2 received normal chow and high fat diet respectively and were administered vehicle. Group 3, 4 and 5 were fed on high fat diet and received 0.25, 0.5 and 1 g kg^{-1} LPG,

respectively. Group 6 and 7 received 0.5 g kg^{-1} LPG plus Propranolol 30 mg/kg/rat and only Propranolol 30 mg/kg/rat, respectively and continued on high fat diet. Group 8 was administered sibutramine 5 mg kg^{-1} along with high fat diet. Vehicle, LPG, Propranolol and Sibutramine were orally administered to the animals by gastric gavage once daily.

Experiment 2: Animals were fed with normal chow or HFD for 8 weeks. Animals fed on normal chow were administered vehicle and continued on same diet for further 8 weeks and were assigned as Group 1. HFD fed animals which showed high body weight gain (obesity) than controls were selected, randomized on the basis of their body weight, divided into ten treatment groups (Group 2 to 11). Group 2 to 6 were employed in experiment 2 and continued on high fat diet for further 8 weeks while as other groups were used in experiment 3. Group 2 received vehicle while as Group 3, 4 and 5 were given LPG 0.25, 0.5 and 1 g kg^{-1}, respectively. Group 6 was administered sibutramine 5 mg kg^{-1}. All the treatments were orally administered to the animals by gastric gavage once daily.

Experiment 3: Animals in Group 7 to 11 received same treatment as Group 2 to Group 6 but the high fat diet was replaced with normal chow diet.

Since, it is not possible to predict which particular animal in the group will develop obesity even when fed HFD and diet control regimens are the first line of non-pharmacological treatment for management of obese patients. These two crucial factors need to be taken into consideration, when designing preclinical studies. For that reason different paradigms were planned in the current study. Thus, animals in the preventive study i.e., experiment 1 imitate the subjects prone to obesity or exposed to obesogenic environment. Subsequently in ameliorative studies, experiment 2 mimics subgroup of obese population with poor compliance/adherence to diet control regimens and experiment 3 mimics obese subgroup with good compliance/adherence to diet control regimens.

Measurements: Food intake and body weight were measured twice weekly. At the end of the stipulated period, blood for various biochemical parameters was obtained from retro-orbital plexuses under light ether anesthesia and the animals were sacrificed by cervical dislocation. The gastrocnemius muscle, epididymal, mesenteric and retroperitoneal white adipose tissue and interscapular brown adipose tissue were dissected, cleaned of, weighed and stored in 10% buffered formalin solution. One week before the sacrifice animals were subjected to Oral Glucose Tolerance Test (OGTT). The blood was collected into tubes, serum separated and analyzed on the same day. Feces of rats were collected on three consecutive days in 7th week for the determination of total fat content. Core body temperature, an index of thermogenesis (Harrold *et al*., 2000) of animals was recorded at the onset of the light phase via rectal route using digital thermometer two days before sacrifice. Animals were familiarized with the procedure 10 days before (except on the day of OGTT) the actual readings. Lee index i.e., $(\text{Body Wt in gms})^{1/3}$ / (ano-nasal length in cm) (Bernardis and Patterson, 1968), an index of obesity, was calculated at the end of the treatments.

Serum glucose, triglyceride, total cholesterol and HDL cholesterol, ALT and AST concentrations were measured by using commercially available kits (Tulip Diagnostic (P) Ltd, Goa, India). During OGTT glucose levels were quantified at the start (t = 0), 30, 60, 90 and 120 min after the administration of the glucose load (2 g kg^{-1}). The total fat content in feces was determined gravimetrically. The samples were dried (105°C for 12 h) and then extracted with petroleum ether under reflux (Malik *et al*., 2011a).

Statistics: All values are expressed as Mean±Standard Deviation (SD). Glucose responses during the glucose tolerance test were evaluated by estimation of the total Area Under the Curve (AUC), using the trapezoidal method. Statistical differences in individual groups for core body temperature before and after fasting were detected using Student's paired t-test. The significance of the differences between the means of control and test animals was established by one way and repeated measures of ANOVA with a Tukey's post hoc test using the Graphpad Prism 4 software. Statistical significance was determined at $p<0.05$.

RESULTS

Phyochecmial screening and determination of main bioactive ingredients: Qualitative phytochemical screening of the LGP showed the presence of bioactive principles such as phenolic compounds, alkaloids, tannins, glycosides, saponins, steroids, flavonoids, carotenoids, ascorbic acid among others. The content of 6-gingerol was found to be 142.3±2.45 mg g^{-1} of LGP. The total phenolic, flavonoids, tannins and ascorbic acid content were estimated to be 7.45±0.91 g gallic acid equivalents/100 g of the LGP, 3.11±0.08 g of quercetin equivalents/100 g of LGP, 2.56±0.06 g tannic acid equivalent/100 g of LGP and 13.8±1.7 mg ascorbic acid/100 g of the LGP, respectively.

Table 1: Effect of Ginger on Body weight, Adipose tissues weight and Various Biochemical Parameters in Rats fed High-Fat Diet for 8 weeks

				Ginger (mg kg^{-1})				
Parameters	NC	HFD-C	Pro per se	250	500	1000	Ginger 1000+Pro	Sibutramine (mg kg^{-1})
Initial body Wt (g)	159±13.8	157.4±18.3	158.4±18.3	159.4±17.3	162.6±13.0	158.1±12.8	161.8±15.2	159.7±11.5
Final body Wt (g)	265±8.2	322.5±36^{a}	326±34.2^{a}	292.4±14^{b}	278.1±15.3^{b}	271.4±15.7^{b}	299.8±14.4^{a}	280.5±21.5^{b}
Lee index	0.268±.01	0.283±0.01^{a}	0.292±0.018^{a}	0.271±0.01^{b}	0.267±0.01^{b}	0.265±0.01^{b}	0.273±0.01^{b}	0.271±0.01^{b}
Body temperature (°F)	98.2±0.5	99.2±0.8^{a}	99.1±0.5^{a}	99.3±0.7^{a}	99.5±0.6^{a}	99.5±0.7^{a}	99.0±0.4^{a}	98.8±0.5^{a}
Energy intake (kcal day^{-1})	64±4.8	79±8.1^{a}	81.1±9.4^{a}	78.4±7.3^{a}	83.2±7.7^{a}	80.5±9.2^{a}	79.8±6.8^{a}	63.1±8.6^{b}
Fecal fat (g day^{-1})	0.06±0.02	0.31±0.03^{a}	0.33±0.03^{a}	0.36±0.04^{b}	0.37±0.04^{b}	0.38±0.04^{b}	0.38±0.03^{b}	0.34±0.04^{a}
WAT (g)	8.9±1.3	21.6±3.6^{a}	20.6±1.1^{a}	15.1±0.9^{b}	12.5±0.5^{b}	10.3±0.6^{b}	14.5±0.9^{b}	14.6±3.2^{b}
Epididymal	2.6±1.3	6.6±1.7	8.3±1.4	5.0±1.1	4.2±0.9	3.6±0.7	4.6±1.1	5.7±1.2
Retroperitoneal	3.3±0.8	8.9±1.7	6.4±1.1	5.4±1	4.8±0.03	3.7±0.7	5.4±1	4.5±1.2
Mesenteric	2.9±0.9	6±0.8	5.9±0.8	4.6±0.5	3.6±0.4	3±0.3	4.6±0.7	4.4±1.6
BAT (g)	0.37±0.2	0.85±0.2^{a}	0.77±0.14^{a}	0.82±0.1^{a}	0.83±0.1^{a}	0.82±0.2^{a}	0.71±0.2^{a}	0.93±0.2^{a}
Gastrocnemius mass (g)	1.9±0.3	2.1±0.2	2.1±0.26	2.1±0.1	2.2±0.9	2.2±0.2	2.1±0.2	2.1±0.2
Glucose (mg dL^{-1})	93.8±20.1	131.6±20.1^{a}	135.8±11.9^{a}	101.8±21.7^{b}	92.1±12.4^{b}	88.3±11.5^{b}	122.7±18.3^{a}	113.8±12.4^{a}
OGTT (AUC_{0-2hr})	14558±1400	18219±2055^{a}	18415±2210^{a}	15663±1105^{b}	15275±1050^{b}	14853±1095^{b}	17702±1515^{a}	16155±1080^{a}
TG (mg dL^{-1})	49.9±13.4	119.8±24.9^{a}	128.3±18.1^{a}	76.7±24.8^{b}	58.1±20.7^{b}	46.4±16.5^{b}	98.1±17.7^{b}	88.6±15.8^{b}
TC (mg dL^{-1})	54.3±11.7	81.6±13.9^{a}	88.5±13.2^{a}	59.6±9.03^{b}	53.2±6.6^{b}	48.7±2.2^{b}	73.5±6.5^{a}	60.4±7.4^{b}
HDL (mg dL^{-1})	23.5±5.1	29.1±4.9^{a}	28.3±7.3^{a}	29.1±4.8^{a}	29.9±5.5^{a}	30.6±5.8^{a}	29.8±9.1^{a}	29.4±6.4^{a}
TC/HDL ratio	2.31±0.3	2.80±0.2^{a}	2.3±0.2^{a}	1.92±0.2^{b}	1.71±0.2^{b}	1.53±0.2^{b}	2.2±0.2^{b}	1.83±0.5^{b}
AST (IU dL^{-1})	67.9±20.5	83.4±13.4	87.3±14.9	75.6±17.6	71.2±16.5	75.3±11.1	72.3±18.2	76.8±13.7
ALT (IU dL^{-1})	32.5±4.5	35.2±9.3	40.8±9.7	38.6±11.7	37.4±7.6	36.9±7.9	39.5±9.3	34.8±6.5

Values are as: Mean±SD. a = $p<0.05$ vs. NC (Normal Control); b: $p<0.05$ vs. HFD-C (High Fat Diet Control); Pro: Propranolol 30 mg/kg/rat

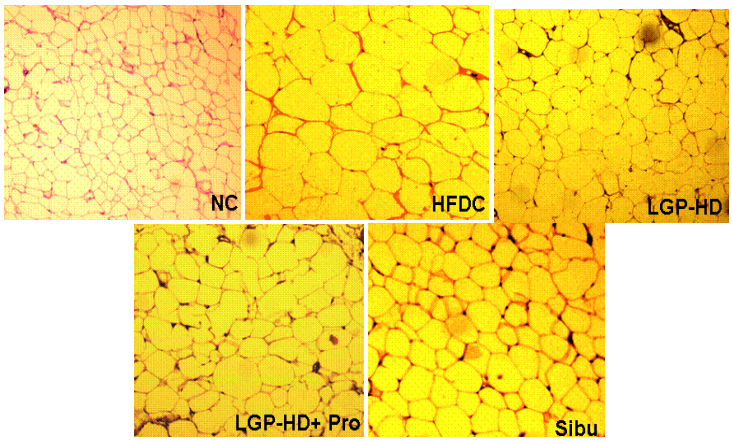

Fig. 1: Histological Changes in Epididymal Adipose Tissue. Representative sections of Hematoxylin-Eosin stained Epididymal Adipose Tissue from Normal chow (NC), High Fat Diet Control (HFD-C), Ginger 1 g kg^{-1} treated animals(LGP-HD), Ginger 1 g kg^{-1} and Propranolol 30 mg/rat treated animals (LGP-HD+Pro) Fed Animals, Sibutramine 5 mg kg^{-1} treated animals (Sibu) (Magnification, X100)

Acute toxicity study: No treatment- related signs of toxicity or mortality in any of the animals were observed. The three doses 250, 500 and 1000 mg kg^{-1} used in the present study correspond to 5, 10 and 20% of no-observed-adverse-effect level (NOAEL) of the LGP (5000 mg kg^{-1}), respectively.

Effect of pharmacological interventions on body weight, adipose tissue weight and lee's index

Experiment 1: Compared with rats fed a normal diet, rats fed a HFD increased their average body weight by 21.5% after 8 weeks of feeding. Parallel to the body weight change, the weights of visceral adipose pads (including epididymal adipose pad, retroperitoneal adipose pad and mesenteric adipose pad) were 142% higher in obese rats than in normal rats. Lee's Index was also significantly increased in HFD fed animals compared to normal chow fed animals. In contrast, HFD induced increase in body weight gain was dose dependently suppressed with LPG treatment (Table 1). LPG treatment also produced dose dependant decrease in visceral adipose pads and Lee's Index. Moreover, histological examination of epididymal WAT revealed that HFD fed rats had markedly increased adipocyte size (Fig. 1) than did normal chow-fed rats. LPG

Table 2: Effect of Ginger on Body weight, Adipose tissues weight and Various Biochemical Parameters in Selected Obese Rats Continued on High-Fat Diet for Further 8 weeks

			Ginger (mg kg^{-1})			
Parameters	NC	OHFD-C	250	500	1000	Sibutramine 5 (mg kg^{-1})
Initial body Wt (g)	272.6±17.8	347.5±20.1	349.2±19.8	350.3±17.9	348.1±19.4	349.6±16.61
Final body Wt (g)	299.5±27.8	431.6±34^{a}	409.8±29	387.8±23.1^{b}	378.7±15.6^{b}	382.6±14.5^{b}
Lee index	0.273±0.01	0.305±0.01^{a}	0.302±0.01	0.297±0.01b	0.288±.0.01^{b}	0.294±0.01^{b}
Body temperature (°F)	98.2±0.4	99.2±0.7^{a}	99.3±0.7^{a}	99.4±0.6^{a}	99.4±0.6^{a}	99.5±0.7^{a}
Energy intake (kcal day^{-1})	69.5±5.6	84±8.7^{a}	81.7±9.3^{a}	85.2±8.2^{a}	86.1±10.5^{a}	60.4±7.4^{b}
Fecal fat (g day^{-1})	0.07±0.02	0.32±0.04^{a}	0.39±0.04^{b}	0.41±0.04^{b}	0.41±0.04^{b}	0.36±0.04^{a}
WAT (g)	10.3±1.9	27.8±2.2^{a}	20.7±1.8^{b}	16.4±1.5^{b}	13.1±1.6^{b}	17.7±2.8^{b}
Epididymal	3.1±1.4	8.8±1.6	6.2±0.8	5.4±0.7	4.3±0.6	6.3±1.8
Retroperitoneal	4.4±1.1	10.6±2.9	7.3±0.9	5.9±0.9	5±0.5	5.8±1.7
Mesenteric	2.8±0.5	8.5±2.2	7.2±0.6	5.1±0.7	3.8±0.7	6.6±1.6
BAT (g)	0.35±0.1	0.93±0.2^{a}	0.88±0.2^{a}	0.9±0.2^{a}	0.88±0.1^{a}	0.9±0.2^{a}
Gastrocnemius mass (g)	2.1±0.2	2.2±0.2	2.3±0.2	2.3±0.2	2.4±0.2	2.2±0.1
Glucose (mg dL^{-1})	96.5±16.7	139.3±21.7^{a}	107.7±16.1^{b}	101.8±21.8^{b}	96.1±16.5^{b}	112.1±9.7^{b}
OGTT (AUC_{0-2hr})	14681±1205	18858±1565^{a}	15644±1175^{b}	15556±1050^{b}	15035±1045^{b}	17692±1146^{a}
TG (mg dL^{-1})	54.3±12.6	132.0±15.8^{a}	90.6±22.7^{b}	75.1±25.4^{b}	62.5±14.9^{b}	107.8±12.8^{b}
TC (mg dL^{-1})	50.9±15.6	86.3±10.1^{a}	66.9±10.2^{b}	61.0±13.3^{b}	58.0±18.7^{b}	72.3±8.6^{b}
HDL (mg dL^{-1})	24.2±6.1	27.9±4.3	29.2±6.5	28.9±6.8	30.1±8.1	29.5±4.5
TC/HDL ratio	2.1±0.2	3.09±0.26^{a}	2.21±0.3^{b}	2.10±0.2^{b}	1.80±0.21^{b}	2.41±0.48^{b}
AST (IU dL^{-1})	73.3±16.6	88.5±10.8	76.6±15.2	77.3±11.6	76.6±16.1	72.4±18.5
ALT (IU dL^{-1})	35.1±5.3	37.1±9.3	39.5±7.9	34.5±6.2	39±8.7	30.8±6.6

Values are as: Mean±SD. a= $p<0.05$ vs NC (Normal Control); b= $p<0.05$ vs OHFD-C (Obese High Fat Diet Control); c= $p>0.05$ vs NC (Normal Control)

or Sibutramine markedly suppressed epididymal adipocyte size compared to high-fat diet fed rats (Fig. 1). However, Propranolol (30 mg/rat/day o.d., p.o.) treatment significantly attenuated LPG induced decrease in body weight gain, adipose pad weight and adipocyte size (Table 1, Fig. 1). The treatment with Propranolol alone for 8 weeks to rats did not produce any significant per se effects on various parameters evaluated in the present study. Sibutramine, the clinically used antiobesity drug, had significant effects not only on reducing body weight and visceral adipose pad weight but also on lowering Lee's Index (Table 1).

Experiment 2: Selected obese rats after 16 weeks of HFD feeding had increased body weight by 44% and visceral adipose pads by 170% than the age matched normal controls. Lee's Index was also significantly increased in obese rats. However, LPG treatment from 9-16 weeks dose dependently attenuated HFD induced increase in body weight, visceral adipose pads weight and Lee's index but the result was not statistically significant for body weight in case of low dose (0.25 g kg^{-1}) of LPG (Table 2). Moreover, administration of sibutramine from 9-16 weeks produced significant reduction in body weight gain, adipose fat pads and Lee's Index in obese rats.

Experiment 3: Body weight of 8 week HFD fed selected obese rats presented NC from 9-16 weeks initially decreased for one week (data not shown) then increased thereafter reaching 12% higher than initial body weight of NC diet presentation. Moreover, even after 8 weeks of NC feeding the obese animals maintained significant higher body weight (28%), adipose tissue weight (131%) and Lee's Index compared to age matched normal rats. However, compared to selected obese rats continued on HFD from 9-16 weeks the final body weight and adipose tissues of selected obese rats presented NC from 9-16 weeks decreased 11% ($p<0.05$) and 14% ($p<0.05$), respectively with concomitant reduction in Lee's Index (Table 3). LPG treatment significantly produced dose dependent decrease in body weight, visceral adipose pads and Lee's Index in selected obese rats fed on NC from 9-16 weeks (Table 3). Sibutramine treatment also produced marked reduction in body weight, visceral adipose pads and Lee's Index in obese rats presented NC diet.

Effect of Pharmacological Interventions on Biochemical Parameters

Experiment 1: HFD for 8 weeks produced significant hyperglycemia (39%), hypertriglyceridemia (137%) and hypercholesterolemia (51%) in rats compared to normal rats (Table 1). TC/HDL ratio was also markedly increased by 8 week HFD feeding. Administration of LGP for 8 weeks markedly reduced HFD induced hyperglycameia, hypertriglyceridemia, hypercholesterolemia and TC/HDL ratio in a dose dependant manner. However, treatment with propranolol significantly prevented LGP produced attenuation of HFD induced hyperglycemia, hypertriglyceridemia and hypercholesterolemia (Table 1). Sibutramine treatment showed significant decrease in serum glucose, triglycerides and total cholesterol concentration as well as marked reduction in TC/HDL ratio.

Table 3: Effect of Ginger on Body weight, Adipose tissues weight and Various Biochemical Parameters in Selected Obese Rats fed Normal Chow Diet for 8 weeks

				Ginger (mg kg^{-1})			
Parameters	NC	OHFD-C	OHFD-NC	250	500	1000	Sibutramine 5 (mg kg^{-1})
Initial Body Wt (g)	272.6±17.8	347.5±20.1	348±18.6	349±17.8	348±19.4	350±18.8	348.1±15.8
Final Body Wt (g)	299.5±27.8	431.6±34^{a}	384.6±19.4*^{a}	338.3±15.8^{b}	328.1±20.7^{b}	318.1±15.6^{b}	305.8±14.5^{b}
Lee index	0.273±0.01	0.305±0.01^{a}	0.296±0.01*^{a}	0.283±0.004^{b}	0.28±0.01^{b}	0.277±0.003^{b}	0.273±0.01^{b}
Body Temperature (°F)	98.2±0.4	99.2±0.7^{a}	98.4±0.4*	98.7±0.6	98.9±0.6	98.9±0.4	98.8±0.7
Energy Intake (kcal day^{-1})	69.5±5.6	84±8.7^{a}	61.2±8.6*^{a}	65.6±7.8*	66.1±9.2*	63.2±9.4*	51.4±7.2*^{b}
Fecal fat (g day^{-1})	0.07±0.02	0.32±0.04^{a}	0.063±0.02*^{a}	0.067±0.03*	0.068±0.02*	0.069±0.02*	0.069±0.01*
WAT (g)	10.3±1.9	27.8±2.2^{a}	23.8±1.4*^{a}	19.6±0.9^{b}	14.5±0.8^{b}	11.6±0.4^{b}	12.8±2.6^{b}
Epididymal	3.1±1.4	8.8±1.6	7.2±1.3	6.5±0.8	4.1±0.6	4.0±0.7	5.4±1.6
Retroperitoneal	4.4±1.1	10.6±2.9	8.8±1.7	7.2±0.9	5.4±1.0	3.8±0.3	3.7±1.7
Mesenteric	2.8±0.5	8.5±2.2	7.8±1.2	5.9±1.0	5.0±0.7	3.9±0.3	3.7±1.3
BAT (g)	0.35±0.1	0.93±0.2^{a}	0.89±0.2^{a}	0.83±0.2^{a}	0.85±0.2^{a}	0.92±0.1^{a}	0.9±0.1^{a}
Gastrocnemius mass (g)	2.1±0.2	2.2±0.2	2.1±0.2	2.3±0.2	2.2±0.1	2.3±0.1	2.1±0.1
Glucose (mg dL^{-1})	96.5±16.7	139.3±21.7^{a}	112.6±18.1*a	92.3±19.1	88.3±15.7	89.7±12.7	97.1±12.7
OGTT (AUC$_{0\text{-}2hr}$)	14681±1205	18858±1565^{a}	17216±1055*^{a}	15541±1125^{b}	15321±1050^{b}	14896±1065^{b}	16276±1321^{a}
TG (mg dL^{-1})	54.3±12.6	132.0±15.8^{a}	85.1±19.7*^{a}	69.5±15.1^{a}	57.8±17.5^{b}	49.6±9.3^{b}	71.2±6.3^{a}
TC (mg dL^{-1})	50.9±15.6	86.3±10.1^{a}	69.1±14*^{a}	55.2±8.4	49.1±9.8^{b}	43.8±8.3^{b}	60.8±8.7
HDL (mg dL^{-1})	24.2±6.1	27.9±4.3	26.1±6.3	25.4±4.7	26.5±3.9	24±4.7	28.5±5.2
TC/HDL ratio	2.1±0.2	3.09±0.26^{a}	2.70±0.2*^{a}	2.16±0.3^{b}	1.85±0.2^{b}	1.82±0.2^{b}	2.15±0.5^{b}
AST (IU dL^{-1})	73.3±16.6	88.5±10.8	79.2±18.8	70.5±13.8	68.5±11.3	67.3±14.5	65.2±7.6
ALT (IU dL^{-1})	35.1±5.3	37.1±9.3	36.1±8.4	35±4.9	34.3±6.8	34.3±6.3	30.4±3.5

Values are as: Mean±SD. * = $p<0.05$ vs OHFD-C (Obese High-Fat Diet Control); a = $p<0.05$ vs NC (Normal Control); b = $p<0.05$ vs OHFD-NC (obese normal chow control)

Experiment 2: Selected obese rats continued on HFD for 16 weeks had 41, 135, 62% higher glucose, triglyceride and total cholesterol levels respectively, than the age matched normal controls (Table 2). TC/HDL ratio was also significantly increased in obese rats compared to normal rats. However, LGP administration from 9-16 weeks significantly attenuated HFD induced hyperglycemia, hypertriglyceridemia, hypercholesterolemia and TC/HDL ratio in obese rats in a dose dependant fashion (Table 2). Sibutramine also reduced HFD induced hyperglycemia, hypertriglyceridemia, hypercholesterolemia and TC/HDL ratio in obese rats.

Experiment 3: 8 week HFD fed selected obese rats when fed on NC from 9-16 weeks showed significant decrease in hyperglycameia, hypertriglyceridemia and hypercholesterolemia compared to selected obese rats fed HFD for 16 weeks but these parameters were still significantly higher than age matched normal control. However, administration of LGP from 9-16 weeks to selected obese rats fed on NC produced dose dependent decrease in hyperglycameia, hypertriglyceridemia and hypercholesterolemia, however, the results were not statistically significant in case of low dose of LGP (Table 3).

Effect of pharmacological interventions on Oral Glucose Tolerance Test (OGTT): There were no significant changes in the fasting glucose concentrations in the normal and HFD control animals indicating HFD used in the present study did not produce frank diabetes even after 16 weeks (data not shown).

Experiment 1: The glucose tolerance ability of the HFD group was significantly lower than that of the NC group. LGP significantly prevented HFD induced glucose intolerance in a dose dependent manner. However, LGP induced better glucose tolerance was significantly attenuated in Propranolol treated animals. Sibutramine treatment also attenuated HFD induced glucose tolerance but did not reach statistically significant level (Table 1).

Experiment 2: Obese rats fed continuously HFD for 16 weeks developed marked glucose intolerance compared to age matched normal controls. However, chronic LGP administration from 9-16 weeks significantly attenuated HFD induced glucose intolerances in a dose dependent manner. Sibutramine treatment also attenuated HFD induced glucose intolerance although insignificantly (Table 2).

Experiment 3: The glucose tolerance ability of selected obese rats presented NC from 9-16 weeks improved significantly compared to selected obese rats continued on HFD for 16 weeks but was still significantly lower than age matched normal rats. LGP treatment to selected obese rats presented NC from 9-16 weeks significantly improved the glucose tolerance ability in a dose dependant manner compared to selected obese rats presented NC (Table 3). Moreover, sibutramine also improved the glucose tolerance ability in selected obese rats presented NC from 9-16 weeks but the result was not statistically significant compared to selected obese rats presented NC (Table 3).

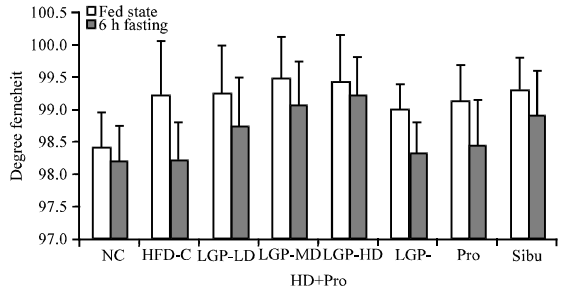

Fig. 2a: Effect of 6 h Fasting on Core Body Temperature in Normal Rats Fed a High-Fat Diet For 8 Weeks. Values are expressed as Mean±SD a = $p< 0.05$ Vs NC in fed state; b = $p<0.05$ vs. animals from same group in fed state. NC: Normal Control; HFD-C: High Fat Diet Control; LGP-LD: Ginger 0.25 g kg^{-1} treated animals; LGP-MD: Ginger 0.5 g kg^{-1} treated animals; LGP-HD: Ginger 1 g kg^{-1} treated animals; LGP-HD + Pro: Ginger 1 g kg^{-1} and Propranolol 30 mg rat^{-1} treated animals; Pro: Propranolol 30 mg rat^{-1} treated animals; Sibu: Sibutramine 5 mg kg^{-1} treated animals

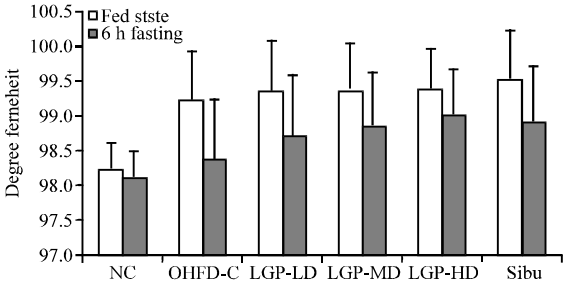

Fig. 2b: Effect of 6 h Fasting on Core Body Temperature In Selected Obese Rats Continued on High-Fat Diet From 9-16 Weeks. Values are expressed as Mean±SD a = $p< 0.05$ vs. NC in fed state; b = $p<0.05$ Vs animals from same group in fed state. NC: Normal Control; OHFD-C: Obese High Fat Diet Control; LGP-LD: Ginger 0.25 g kg^{-1} treated animals; LGP-MD: Ginger 0.5 g kg^{-1} treated animals; LGP-HD: Ginger 1 g kg^{-1} treated animals; Sibu: Sibutramine 5 mg kg^{-1} treated animals

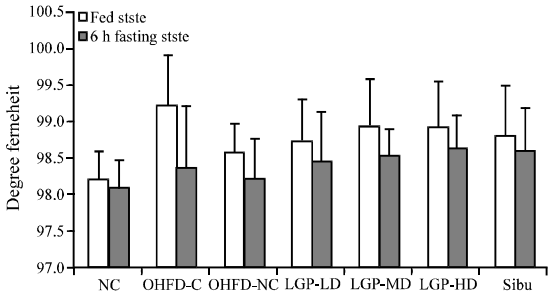

Fig. 2c: Effect of 6 h Fasting on Core Body Temperature in Selected Obese Rats Fed Normal Chow Diet from 9-16 Weeks. Values are expressed as Mean±SD * = $p<0.05$ Vs OHFD-C in fed state; a = $p<0.05$ vs. NC in fed state b = $p<0.05$ vs. animals from same group in fed state. NC: Normal Control; OHFD-C: Obese High Fat Diet Control; OHFD-NC: Obese Normal Chow Diet Control; LGP-LD: Ginger 0.25 g kg^{-1} treated animals; LGP-MD: Ginger 0.5 g kg^{-1} treated animals; LGP-HD: Ginger 1 g kg^{-1} treated animals; Sibu: Sibutramine 5 mg kg^{-1} treated animals

Effect of pharmacological interventions on core body temperature

Experiment 1: Core body temperature was significantly higher in HFD fed rats than normal rats. Animals on LGP and sibutramine treatment had slightly but insignificantly higher core body temperature compared to HFD control. However, Propranolol treatment prevented LGP induced increase in core body temperature in HFD rats. Moreover, when feed was removed for six hours the core body temperature of HFD group decreased significantly but not in case of NC animals. However, this fall in core body temperature was not significant in LGP and sibutramine treated animals indicating sustained thermogenic effect. Propranolol treatment, however, prevented LGP induced sustained thermogenic effect in fasting state in HFD rats (Fig. 2a).

Experiment 2: Core body temperature was significantly higher in HFD induced obese rats than normal rats. Treatment with LGP further enhanced it, however, insignificantly compared to HFD control. Moreover, when feed was removed in HFD control group for six hours the core body temperature of HFD group decreased significantly. However, this fall in core body temperature was not significant in LGP treated animals (Fig. 2b).

Experiment 3: When HFD was replaced with NC in selected obese rats the core body temperature showed slight decrease compared to selected obese rats continued on HFD for 16 weeks but was still slightly higher than the age matcher normal rats. However, ginger or sibutramine treatment in selected obese rats continued on NC slightly but insignificantly increased the core body temperature compared to the obese rats presented NC. Selected obese rats with or without treatment continued on normal chow when fasted for 6 h did not show significant fall in core body temperature (Fig. 2c).

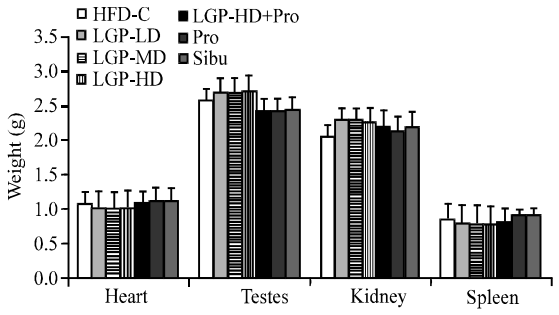

Fig. 3: Effect of Pharmacological Interventions on Weight of Various Tissue in Normal Rats Fed High-Fat Diet For 8 Weeks. Values are expressed as Mean±SD HFD-C: High Fat Diet Control; LGP-LD: Ginger 0.25 g kg^{-1} treated animals; LGP-MD: Ginger 0.5 g kg^{-1} treated animals; LGP-HD: Ginger 1 g kg^{-1} treated animals; LGP-HD+Pro: Ginger 1 g kg^{-1} and Propranolol 30 mg/rat treated animals; Pro: Propranolol 30 mg/rat treated animals; Sibu: Sibutramine 5 mg kg^{-1} treated animals

Effect of pharmacological interventions on average daily energy intake and fecal fat content: Average daily energy intake and fecal fat content of rats on HFD groups was significantly increased compared to normal rats. However, ginger treatment did not affect average daily energy intake but significantly decreased fecal fat content of rats on HFD indicating decrease in apparent fat absorption (Table 1). This decrease in apparent fat absorption was not observed when obese rights were switched to normal chow diet suggesting decreased fat absorption or increased fat excretion in high fat diet conditions and not in normal condition. Sibutramine treated groups, however, exhibited significant reduction in average daily energy intake but not on fecal fat content compared to corresponding HFD groups indicating sibutramine decreases feed intake but has no effect on fat absorption.

Effect of pharmacological interventions on liver function and other organs: In all the experiments no significant changes in the AST and ALT levels were observed in normal, HFD fed or treatment groups suggesting that liver function was not significantly altered. Furthermore, LGP or sibutramine treatment did not produce any significant change in gastrocnemius muscle weight, an index of lean tissue mass, indicating LGP or sibutramine promotes loss of fat rather than lean tissue. HFD significantly increased the brown adipose issue compared to the normal rats which was not reduced with LGP, sibutramine or propranolol treatment. Moreover, no change in weight of different organs in HFD groups was observed (Fig. 3) suggesting treatments were devoid of any toxicity.

DISCUSSION

In the present study, the antiobesity effect of Lyophilized Ginger Powder (LGP) in HFD induced obesity in wistar rats was examined. LGP not only reduces body weight gain, Lee's index, adipose tissue weight but also improves glucose and lipid homeostasis and decreases TC/HDL ratio. These beneficial effects were attenuated by propranolol indicating the role of beta-adrenoceptors. Further investigation showed that LPG treatment was also effective in ameliorating established obesity with associated biochemical consequences and more so when HFD was replaced with normal diet indicating the importance of dietary modification in successful obesity therapy. The result suggests that LGP has a potential role in therapy for obesity and associated disorders. To our knowledge, this is the first study to investigate the preventive as well as ameliorative effects of LGP on body weight, visceral adipose tissues and glucose and lipid homeostasis in HFD-induced obese rat model along with dietary modifications.

The consumption of HFD leads to obesity, accumulation of body fat and disturbance in glucose and lipid homeostasis which is consistent with our recent studies and reports by others (Dreon *et al*., 1988; Malik *et al*., 2011b). Dietary fat being calorically dense and extremely palatable is easily over consumed because it causes less satiety than carbohydrate and protein (Rolls and Hammer, 1995). Moreover, ingestion of fat does not acutely stimulate fat oxidation or thermogenesis but rather, it promotes fat storage (Rolls and Hammer, 1995). Moreover, a HFD not only lowers glucose uptake but also inadequately suppresses hepatic glucose production stimulated by insulin leading to insulin resistance as well as hyperglycaemia (Oakes *et al*., 1997). HFD also alters both basal and stress induced hypothalamic pituitary adrenal activity to increase adrenal glucocorticoid production in rodents (Tannenbaum *et al*., 1997). Elevated glucocorticoids can subsequently lead to hypertriglyceridemia by decreasing the level of lipoprotein lipase (Mantha *et al*., 1999). Therefore, a HFD can lead to hyperphagia, weight gain, increased adiposity and disturbance in glucose and lipid homeostasis. Thus, the observed obesity and subsequent biochemical disturbances in HFD animals may be due to hyperphagia, inefficient diet induced thermogenesis and fat storage. This contention is supported by the results obtained in the present study that compared to normal animals HFD animals had higher average daily energy intake without significant increased in core body temperature (an index of thermogenesis) and increased adipose tissue weight.

An increase in the size of the adipocyte also supports the debate that HFD induces fat accumulation.

Treatment with LGP significantly attenuated body weight gain both in preventive and ameliorative studies which was accompanied by reduction of various visceral adipose tissues including mesenteric, retroperitoneal and epididymal White Adipose Tissue (WAT). The observed antiobesity effect of ginger is in accordance with earlier reports (Han *et al*., 2005; Nammi *et al*., 2009). Further, it was interesting to note that after LGP treatment in ameliorative studies the body weight and adipose tissue weight of obese rats fell below the initial values when the HFD was replaced with NC suggesting comprehensive structured dietary plan can enhance weight loss when given in conjunction with pharmacotherapy. This observation is supported by the clinical reports that pharmacotherapy alone is not as effective in obese patients as pharmacotherapy along with dietary modifications (Wadden *et al*., 2004). Moreover, it is well documented that basal metabolic rate and core body temperature are generally regarded as the outcome of a regulatory process that regulate cellular energy utilization (thermogenesis) and are known to be primarily under the control of the sympathetic nervous system (Webber and Macdonald, 2000; Weyer *et al*., 2000). Enhanced thermogenesis by increasing sympathetic activity results in expenditure of excess energy as heat, increased mobilization of WAT i.e. lipolysis and increased fat oxidation in Brown Adipose Tissue (BAT) subsequently leading to weight loss without decreased food intake (Weyer *et al*., 1999). Ginger is reported to raise body temperature in humans (Fujisawa *et al*., 2005) and promotes thermogenesis in rats (Eldershaw *et al*., 1992). Moreover, ginger constituents are reported to increase the adrenaline secretion which may be responsible for the increased thermogenesis by acting on beta-adrenoceptors (Iwasaki *et al*., 2006). Interestingly, Pulbutr *et al*. (2011) recently reported that ginger constituents can induce lipolysis in HFD derived adipocytes. So it is possible to speculate that LGP enhanced the thermogenesis by increasing the sympathetic activity via beta-adrenoceptors which consequently increased WAT lipolysis and resulted decrease in body weight and WAT weight. This is supported by the observation that LGP induced rise in core body temperature and antiobesity action was attenuated by nonspecific beta-adrenoceptor blocker, propranolol. The decreases in body weight may be attributed to decrease in body fat rather than lean tissue. Both the changes in the weight of the visceral fat pads and no significant change in gastrocnemius muscle weight are supportive of this interpretation. A decrease in the adipocyte size further support the argument that LGP suppresses HFD induced adipose tissue accumulation.

Sibutramine, central reuptake inhibitor of serotonin and noradrenaline, has been shown to cause weight loss in humans and rodents (Connoley *et al*., 1999). Sibutramine increases core body temperature by stimulating thermogenesis by increasing sympathetic activation of BAT thermogenesis via beta-adrenoceptors (Connoley *et al*., 1999). Therefore, in the present study, sibutramine has been employed as a standard drug to compare the potential of LGP in preventing HFD induced weight gain. The beneficial effect of LGP in attenuating the HFD induced weight gain has been observed to be almost similar to that produced by sibutramine. However, unlike sibutramine LGP did not affect the feed intake suggesting different mechanism or site of action from sibutramine and needs further investigation.

Moreover, LGP treatment also decreased the HFD induced hyperglycemia and improved glucose tolerance. The blood glucose lowering effect of ginger reported in number of experimental models (Al-Amin *et al*., 2006; Al-Attar and Zari, 2007) substantiate our findings in the HFD fed rats. The hypoglycaemic action of ginger may be due to effects involving serotonin receptors, an increase in pancreatic secretion of insulin from beta cells or release of bound insulin (Al-Amin *et al*., 2006). Changing diet from high to low fat has also been reported to increase insulin sensitivity (Franssila-Kallunki *et al*., 1992). This may explain better glucose tolerance observed in selected obese rats fed with normal chow.

Treatment with LGP also decreased HFD induced elevated lipid levels. The reduction in elevated lipid levels is supported by earlier reports (Bhandari *et al*., 1998). The hypocholesterolemic (hypolipidemic) effect observed after consumption of LGP could have possibly resulted, at least in part, by conversion of cholesterol to bile acids (Srinivasan and Sambaiah, 1991) and/or from the inhibition of cellular cholesterol biosynthesis (Fuhrman *et al*., 2000). Reduced cellular cholesterol biosynthesis is coupled with increased activity of the LDL receptor, which in turn leads to enhanced removal of LDL from plasma, resulting in reduced plasma cholesterol concentration (Neess *et al*., 1996). So it is possible to hypothesize that in the present study LGP stimulates the cholesterol excretion in HFD rats which result in decreased plasma lipid levels. This contention is supported by the results obtained in the present study that treatment with LGP significantly enhanced the fecal fat content in HFD rats. Further, the observation in the present study that LGP significantly decreased the TC/HDL ratio which is considered the best single lipid predictor of coronary artery disease (CHD) risk (Lewington *et al*., 2007) is supported by the reports that ginger consumption is associated with lower risk of heart diseases (Nicoll and Henein, 2009).

CONCLUSION

From the above discussion it can be concluded that ginger can prevent as well as ameliorate obesity and improve associated glucose-lipid anomalies. These beneficial effects seem to have many components. Thus the insulin sensitizing activity, interference with adipose tissue metabolism, increased sympathetic activity induced thermogenesis all may contribute to the antiobesity activity of ginger. This is significant as obesity is a multifactorial disorder and combination therapies have proven better than single therapy (Greenway *et al*., 2009). Moreover, the marked suppression of TG, abdominal fats and TC/HDL ratio three well reported risk factors of cardiovascular disease (Bergman *et al*., 2007), advocate its use in obese patients in general and obesity related cardiovascular patients in particular.

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