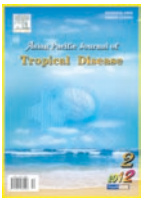
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd



Document heading doi:

Synergistic effect of *Commiphora mukul* (gum resin) and *Lagenaria siceraria* (fruit) extracts in high fat diet induced obese rats

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ARTICLE INFO

Article history:
Received 15 August 2012
Received in revised from 15 September 2012
Accepted 17 December 2012
Available online 28 December 2012

Keywords:
Commiphora mukul
Lagenaria siceraria
High fat diet induced obesity
Lipid profile

ABSTRACT

Objective: To investigate the synergistic effect of *Commiphora mukul* (gum resin) and *Lagenaria siceraria* (fruit) extracts in high fat diet induced obese rats. **Methods:** Rats were randomly divided into seven groups: (i) non-obese control (NOB), (ii) Obese control (OB), (iii) orlistat (50 mg/kg; *p.o.*), (iv) ethyl acetate extract of *Commiphora mukul* (gum resin) (200 mg/kg; *p.o.*), (v) ethanolic extract of *Lagenaria siceraria* (fruit) (200 mg/kg; *p.o.*) were examined individually, (vi) *C. mukul* and *L.siceraria* (200 mg/kg; *p.o.*) and (vii) *C. mukul* and *L.siceraria* (400 mg/kg; *p.o.*)extracts were administered in combination to the high fat-diet-induced obese rats for 30 days to evaluate its synergistic activity. **Results:** For synergistic effect, after combination treatment caused most significant ($P<0.001$) reduction in body weight, fasting blood glucose, serum levels of cholesterol, triglyceride, LDL, VLDL and increase levels of HDL. **Conclusions:** The result demonstrated that combination *C.mukul* and *L.siceraria* has ameliorated the high fat diet induced obesity.

1. Introduction

Obesity is characterized by an increase in the number and size of adipocytes differentiated from fibroblastic preadipocytes in adipose tissues[1]. Therefore, a detailed understanding of the mechanisms that govern the balance between lipid deposition and lipid mobilization is fundamentally important for the treatment of obesity. It has been reported that less than 5% of triglyceride (TG) are synthesized in liver and adipose tissue themselves. Most free fatty acids (FFAs) that are stored as TG in adipose tissue are derived from the diet[2]. Pancreatic lipase is important for the digestion and absorption of fat. Lipoprotein lipase, which is secreted by a variety of cell types, is an indicator of the anabolic pathway that provides FFAs to the adipocytes directly from the plasma lipoproteins and indirectly from the diet. Recently it has been reported that high fat diets are responsible for high global prevalence of obesity[3, 4].Therefore, agents that regulate food intake and food absorption target the first important step in the process of body fat regulation. There is overwhelming evidence that obese individual have a substantially higher risk of developing many diseases such as type II-diabetes, hyperlipidemia, cardiovascular disease and hypertension[5].

In the present study an attempt was made to establish the synergistic effect of ethyl acetate extract of *Commiphora mukul* (gum resin) and ethanolic extract of *Lagenaria siceraria* (fruit) in high fat diet induced obese rats. *C.mukul* (gum resin) and *L.siceraria* (fruit) extracts were used in combination against obesity because some time individual plant extract is active only at higher doses which are economically less beneficial for use. Phytochemical analysis reveals the presence of bio-active compounds responsible for antiobesity effect. Therefore, the research was conducted to evaluate synergistic effect of *C.mukul* (gum resin) and *L.siceraria* (fruit) extracts in high fat diet induced obese rats.

2. Materials and methods

2.1. Animals

Albino wistar rats of either sex weigh between 150 and 180 g were used for the present study. Young healthy rats were housed in opaque polypropylene cages (28×21×14 cm) and maintained at 23±2℃ under 12:12 h light/dark cycle (0700-1900 h) with free access to rodent chow and tap water. The animal studies were approved by the Institutional Animal Ethics Committee (Reg. no. 784/03/C/CPCSEA/5) constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests,

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2.2. Plant material and Chemicals

Ethyl acetate extract of *Commiphora mukul* (voucher specimen GG-25) and ethanolic extract of *Lagenaria siceraria* (voucher specimen LP-09011) Ambe Phyto Extract Ltd. used in present study were dissolved in distilled Water. Orlistat (Lipocut 50), glucose estimation kit (Crest Biosystems, India), triglyceride estimation kit (Crest Biosystems, India), HDL estimation kit (Crest Biosystems, India), total cholesterol estimation kit (Crest Biosystems, India) were used. Other chemicals and reagents used for the study were of analytical grade.

2.3. Phytochemical analysis of plant extracts

Phytochemical analysis of the plant extracts were carried out according to the method of Siddiqui, 2011 as well as Trease and Evans, 2002 to get an assumption on active ingredient responsible for antiobesity effect. Phytochemical analysis revealed that the presence of terpenes, myrcene, eugenol, phenylpropanoids, Z-guggulsterone, E-guggulsterone in the *C.mukul* (gum resin) extract[6] and flavonoids, saponins, steroids, polyphenolics compound in the *L.siceraria* (fruit) extract[7].

2.4. Acute toxicity study

Acute toxicity study was carried out for the combination of *C.mukul* and *L.siceraria* extracts following OECD guidelines[8]. The extracts were dissolved in distilled water. The dose of 2 g/kg body weight was orally administered to overnight-fasted, healthy rats (n = 6).The animals were observed continuously for 24 h for mortality.

2.5. Induction of experimental obesity and treatment protocol

Animals were randomly divided into seven groups, each group consisting of 6 animals.
Group I: Non-obese (NOB).
Group II: Obese Control (OB).
Group III: (OB + Orl): obese standard treated, 50 mg/kg of orlistat *p.o.*
Group IV: (OB + CM): obese *C. mukul* extract 200 mg/kg; *p.o.*
Group V: (OB + LS): obese *L.siceraria* extract 200mg/ kg; *p.o.*
Group VI: (OB + COMB): obese *C. mukul* and *L. siceraria* extracts 200mg/ kg; *p.o.*
Group VII: (OB + COMB): obese *C. mukul* and *L. siceraria* extracts 400mg/ kg; *p.o.*

The method described by HU and Davies (2009) was done with suitable modification in the study[9].Group I was served as non-obese control (NOB) and fed with normal diet throughout the course of study. Groups II to VII were fed with high-fat diet for 30 days. Group III was fed high fat diet and treated with orlistat (50mg/kg; *p.o.*) suspension prepared with saline. Animals of group IV to V were received high fat diet and alone *C. mukul* and *L. siceraria* extract in doses of 200mg/kg; *p.o.* and groups VI to VII were received high fat diet and combined extracts of *C. mukul* (CM) and *L. siceraria* (LS) in divided doses of 200 and 400 mg/kg; *p.o.* respectively in addition for next 30 days.

2.6.1. Estimation of plasma glucose, body weight and lipid profile

The plasma glucose estimation was done by the glucose oxidase/peroxidase (GOD/POD)[10] using a standard kit obtained from Crest Biosystems, India. Body weight of all experimental animals were recorded using a digital weighing scale. Lipid profiles of all the rats were determined on 30 days (post-treatment).

2.6.2. Estimation of biochemical parameters

The biochemical parameters were determined after 30 days of treatment in all rats. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalate transaminase (SGOT)[11] were estimated by using standard kits obtained from Crest Biosystems, India.

2.7. Statistical analysis

The results are expressed as a mean ± S.E.M. Data were analyzed using one-way analysis of variance (ANOVA) after Tukey's multiple comparison tests. P<0.05 was considered statistically significant in all the cases.

3. Results

3.1. Effect of C. mukul and L. siceraria extracts on fasting blood glucose

The blood glucose level in obese control animals exhibited increased fasting blood glucose significantly when compared with non-obese control animals. Combined extracts of *C. mukul* and *L. siceraria* (200 mg/kg, 400 mg/kg) and orlistat 50 mg/kg *p.o.* showed a significant (P<0.001) reduction fasting blood glucose as compared with obese control animals. Treatment with CM and LS extract (200 mg/kg/p.o) exhibited significant (P<0.01) marked reduced when compared with obese control animals (Table 1).

3.2. Effect of C. mukul and L. siceraria extracts on body weight

Body weight of OB group was found to be significantly (P<0.001) increased compared to non-obese control rats (Table 2). After first week of treatment with combined extracts of *C. mukul* and *L. siceraria*, and orlistat the body weight significantly (P<0.001) reduced compared to obese control rats. Reduction in weight gain of animals in combined and standard-treated group was continued to be observed till the end of the study (Table 1).

3.3. Effect of C. mukul and L. siceraria extracts on lipid profile

One-way ANOVA indicated that levels of serum total

cholesterol and triglycerides were increased in all the obese groups. Treatment with combined extracts of *C. mukul* and *L. siceraria*, and orlistat significantly ($P<0.001$) decreased the elevated total cholesterol and triglycerides from the first week of treatment onwards and this effect was observed throughout till the end of the study (Table 2). Similarly, serum VLDL and LDL levels were raised in the obese control group, which was effectively ($P<0.001$) reduced by treatment with combined extracts of *C. mukul* and *L. siceraria* from first week of treatment onwards till the end of the study (Table 2). Treatment with alone CM and LS (200 mg/kg/p.o) caused significant ($P<0.01$, $P<0.05$) decreased in cholesterol, triglycerides, VLDL and LDL as compared with obese control animals.

Serum HDL levels were decreased in all obese animals. These levels were brought to normal by treatment with combined extracts of *C. mukul* and *L. siceraria* within second week of treatment and the effect continued till the end of the study (Table 2).

3.4. Effect of C. mukul and L. siceraria extracts on biochemical parameters

SGPT and SGOT levels were significantly increased in obese rats, which was significantly ($P<0.001$) reduced by treatment with combined extracts of *C. mukul* and *L. siceraria*, and orlistat 50 mg/kg *p.o.* from first week of treatment onwards till the end of the study (Table 3).

Table 1

Effect of *C. mukul* and *L. siceraria* extracts on body weight and fasting blood glucose levels in high fat diet induced obese rats.

Group	Treatment	Body weight (g)		Blood glucose (mg/dl)	
		Onset of study	End of study	Onset of study	End of study
NOB control	Vehicle (1ml/kg)	150.2 ± 3.27	167.8 ± 4.62	86.23 ± 5.16	84.00 ± 6.03
OB control	HFD	178.3 ± 4.56	268.5 ±9.01#	98.33 ± 4.03	157.5 ± 3.21#
Orlistat	Orl (50 mg/kg)	164.7 ± 5.23	171.5 ± 1.64***	95.35 ± 5.84	90.33 ± 3.92***
OB + CM	CM (200 mg/kg)	178.6 ± 7.56	216.7 ± 4.06*	96.00 ± 6.24	116.0 ± 7.37**
OB + LS	LS (200 mg/kg)	171.0 ± 4.78	209.2 ± 2.38 **	89.23 ± 4.08	115.7 ± 6.66**
OB + COMB	COMB (200 mg/kg)	169.4 ± 8.90	179.7 ± 5.63***	84.00 ±5 .74	94.50 ± 4.03***
OB + COMB	COMB (400 mg/kg)	168.3 ± 6.87	174.2 ± 7.52***	94.5 ± 5.76	91.67 ± 3.58***

CM: *C. mukul* (200 mg/kg), LS: *L. siceraria* (200 mg/kg), Orl: Orlistat (50mg/kg), COMB: Combined extracts of *C. mukul* and *L. siceraria* (200mg/kg, 400 mg/kg).Data are expressed as Mean ± S.E.M; $n = 6$. ANOVA followed by Tukey's post hoc test. Values are statistically significant at # $P<0.001$ vs. non-obese group; ***$P<0.001$, **$P<0.01$, *$P<0.05$ vs. obese control group.

Table 2

Effect of *C. mukul* and *L. siceraria* extracts on serum lipid profile in high fat diet induced obese rats.

Groups	Treatment	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
NOB control	Vehicle (1ml/kg)	77.5 ± 3.57	177.4 ± 3.59	27.4 ± 2.40	32.1 ± 2.39	40.9 ± 1.40
OB control	HFD	148.4 ± 3.43#	239.3 ± 1.54#	59.57 ± 1.76#	50.8 ± 1.31#	31.2 ± 2.28ns
Orlistat	Orl (50 mg/kg)	89.41 ± 1.85 ***	185.3 ± 3.01 ***	37.8 ± 4.18***	37.6 ± 1.04***	41.3 ± 1.23***
OB + CM	CM (200 mg/kg)	134.0 ± 6.21**	214.3 ± 2.02*	49.6 ± 3.69**	44.5 ± 0.540*	36.9 ± 2.31*
OB + LS	LS (200 mg/kg)	139.3 ± 4.46*	213.2 ± 1.50*	51.4 ± 1.51*	43.8 ± 0.170*	35.6 ± 1.13*
OB + COMB	COMB (200 mg/kg)	97.9 ± 3.91 ***	193.2 ± 3.70 ***	39.9 ± 1.17***	40.5 ± 1.24***	40.1 ± 1.73**
OB + COMB	COMB (400 mg/kg)	92.3 ± 3.85 ***	188.6 ± 3.05 ***	38.2 ± 1.92***	39.1 ± 1.12***	41.8 ± 2.35***

CM: *C. mukul* (200 mg/kg), LS: *L. siceraria* (200 mg/kg), Orl: Orlistat (50 mg/kg), COMB: Combined extracts of *C. mukul* and *L. siceraria* (200 mg/kg ,400 mg/kg).Values are expressed as mean ± S.E.M. (n=6).Values are statistically significant at # $P<0.001$, ns-nonsignificant ($P>0.05$) vs. nonobese group; ***$P<0.001$, **$P<0.01$, *$P<0.05$ vs. obese control group respectively (One-way ANOVA followed by Tukey's post hoc test).

Table 3

Effect of *C. mukul* and *L. siceraria* extracts on biochemical parameters in high fat diet induced obese rats.

Groups	Treatment	SGOT (IU/L)	SGPT (IU/L)
NOB control	Vehicle (1ml/kg)	29.32 ± 0.53	22.27 ± 0.85
OB control	HFD	53.17 ± 1.20#	44.56 ± 0.99#
Orlistat	Orl (50 mg/kg)	34.01 ± 1.10***	29.89 ± 0.98***
OB+CM	CM (200 mg/kg)	41.35 ± 0.95*	41.27 ± 0.50*
OB+LS	LS (200 mg/kg)	40.34 ± 0.62*	38.86 ± 0.33*
OB + COMB	COMB (200 mg/kg)	39.01 ± 0.55**	33.53 ± 0.88***
OB + COMB	COMB (400 mg/kg)	37.73 ± 0.51***	31.70 ± 0.92***

CM: *C. mukul* (200 mg/kg), LS: *L. siceraria* (200 mg/kg), Orl: Orlistat (50 mg/kg) , COMB: Combined extracts of *C. mukul* and *L. siceraria* (200 mg/kg ,400 mg/kg).Values are expressed as mean ± S.E.M. (n=6).Values are statistically significant at # $P<0.001$ vs. nonobese group; ***$P<0.001$, **$P<0.01$, *$P<0.05$ vs. obese control group respectively (One-way ANOVA followed by Tukey's post hoc test).

4. Discussion

Feeding rats high-fat diet causes hyperphagia resulting in increased body weight compared to pellet chow fed animals and is a widely accepted model for clinical obesity. The body weight gain is largely due to increased fat mass as a result of preadipocytes proliferation and differentiation and, to an extent, accumulation of lipids in the liver[12, 13].

In the present study crude mixed extracts of *C. mukul* and *L. siceraria* at combination were found to be the best combination when compared to other combinations. Our results demonstrated that the combination has pronounced synergistic effect in high-fat diet-induced obese rats. These effects were reflected in the body weight, liver functioning test, fasting plasma glucose and serum lipid profiles of the rats in our various treatment groups.

These results suggest that crude mixed extracts of *C. mukul* and *L. siceraria* may be decreased the fasting plasma glucose (Table 1) and reduce weight gain induced by a high fat diet; it seemed that low body weight in combined groups partially due to loss of appetite.

Treatment with *C. mukul* extract alone or with *L. siceraria* supplementation has manifest decreasing the concentration of serum TG, TC, LDL, VLDL and of increasing the ratio of HDL. These results are in agreement with previous results[14-17] and suggested that combined supplementation of *C. mukul* and *L. siceraria* extracts showed better blood lipid profiles compared to *C. mukul* and *L. siceraria* extract alone. Thus, the lowered cholesterol concentrations after crude mixed extracts *C. mukul* and *L. siceraria* supplement, resulted from decreased lipid digestibility and enhanced fecal cholesterol excretion. Diet-induced obesity is the most relevant stimulus for the induction of atherosclerosis lesions in human[18].

Several studies shown that biochemical parameters did not show any of the adverse effect of crude mixed extracts on experimental animals. Liver enzymes such as SGOT and SGPT are considered to be biochemical markers for assessing liver function. Hepatotoxicity is evidenced by an elevation of the serum marker enzymes[19]. The combined extracts of *C. mukul* and *L.siceraria* significantly ($P<0.001$) reduced the liver enzymes levels in experimental animals shows that combined therapy has hepatoprotective action. During the experimentation, Wistar rats did not show any mortality or any other adverse effects when the rats fed orally with combined extracts of *C.mukul* and *L.siceraria* at the doses of 200-400 mg/kg/day. Thus the combined extracts of *C. mukul* and *L.siceraria* has a good margin of protection.

In conclusion, the combined extracts of *C. mukul* (gum resin) and *L.siceraria* (fruit) show pharmacological potential against high fat diet induced obesity due to synergism of plant extracts.

Acknowledgements

The author would like to thank TIT group of institution, Bhopal and Dr. Rana Noor Siddiqui, USA. for helpful suggestions.

Conflict of interest

No conflict of interest.

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