International Food Research Journal 23(3): 1115-1122 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Strobilanthes crispus leaves extracts (SCE) induced lipolysis and increased leptin level in diet-induced obese rats fed high-fat diet

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Article history

Received: 22 July 2015
Received in revised form: 27 September 2015
Accepted: 6 October 2015

Keywords

Strobilanthes crispus
Adipose tissue
Lipolysis
Leptin

Abstract

The aim of the present study was to assess the anti-obesity and lipolysis effects of *Strobilanthes crispus* leaves extract (SCE) in diet-induced obese (DIO) rats by administering 1% (w/w) of SCE in drinking water. Methods: Normal Sprague-Dawley rats were induced obese using a high-fat (HF) diet formulation for 14 weeks. DIO rats were subsequently treated with 1% (w/w) SCE while the HF diet was switched to normal rat chow diet. Food intake, water intake and bodyweight were measured weekly. Other parameters e.g. blood lipid profile were determined in normal and DIO rats before and after treatment with SCE. Histopathological changes in the liver were also observed after treatment. Results: Prior to treatment, DIO rats have significantly higher ($p < 0.05$) bodyweight, adipose tissue and liver weight, plasma leptin levels, lower adipose tissue lipolysis rate and severe fatty livers and the trend continues in non-treated DIO rats served as Control group. DIO rats treated with SCE significantly ($p < 0.05$) reduced their bodyweight gain, have lower adipose tissue and liver weight, lower leptin level and increased lipolysis rate although no significant effect was found in total bodyweights of treated group. Lower glucose level and improved state of fatty liver was also noted in SCE treated group. The observed anti-obesity effects are most likely the due to lipolysis. Conclusion: SCE may, at least in part, be attributed to the anti-obesity effects found on treated DIO rats.

Introduction

Obesity prevalence is increasing worldwide at an alarming rate in both developed and developing countries. Obesity has reached epidemic proportions globally, and all this evidence suggests that the situation is likely to get worse. Obesity is now epidemic in developed nations including Australia, New Zealand and Singapore and is rapidly becoming so in many developing populations, particularly Pacific Islanders and certain Asian nations (WHO, 1998). Obesity involves complex aetiological links between the genetic, metabolic and neural frameworks on one hand and behavior, food habits, physical activity and socio-cultural factors on the other (Nammi *et al*., 2004).

In the past a variety of drugs that influence appetite, energy expenditure, or both were used; however, all have been found to cause serious side effects and are considered inappropriate for the pharmacological treatment of obesity (Acheson and Tappy, 2004). For example, Rimonabant, a selective blocker of the cannabinoid receptor CB1 is associated with psychiatric problems (mainly depression and anxiety) and the marketing authorization of the relevant drug was suspended by the European Medicines Agency (EMA) in 2008. Sibutramine, a centrally acting agent that inhibits serotonin and norepinephrine reuptake; marketing authorization of the relevant drugs was suspended by EMA in 2010 due to association with increases in heart rate and blood pressure. Finally, orlistat, a triacylglycerol lipase inhibitor that works in the intestinal lumen to reduce dietary fat absorption produces gastrointestinal side effects (as abdominal pain, fecal urgency etc) in 15–30% of the patients under treatment (Fragkiadakis *et al*, 2010).

Research studies are being carried out to detect and confirm the action of drugs and natural products that yield better and long-lasting results in terms of weight reduction (Gurib-Fakim, 2006). Plants or medicinal herbs can contribute to prevent or treat obesity through many factors including inhibition of lipases, increased thermogenesis, increased satiety and reduced stress. Some medicinal plant preparations can be usefully associated to diet therapy (Gurib-Fakim, 2006).

The biochemical properties of *S. crispus* show the potential for anti-obesity treatment. The plant is

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native to countries from Madagascar to Indonesia, and commonly known as daun picah beling in Indonesia (Sunarto, 1977). This bush-like plant can be found on riverbank or abandoned field while some Javanese use this plant as fence. Previous research has found the plant to have antidiabetic, diuretic, antilytic, and laxative effects (Perry and Metzger, 1980). One study found that the main active compounds of *S. crispus* are phenolic acids (Sergent *et al*., 2012). Phenolic compounds have now been shown to have the capacity of preventing obesity (Maznah *et al.*, 2000). The plant also has many cystoloths of calcium carbonate and an infusion is mildly alkaline (Perry and Metzger, 1980). High levels of total ash (21.6%) in the dried plant leaves were the result of high content of minerals such as potassium (10,900 mg/100 g sample), followed by calcium (5,185 mg/100 g sample) (Zemel, 2002). It has been proposed that dietary calcium regulates energy metabolism and lipolysis (Xue *et al*., 2001) by modulating intracellular phosphodiesterase and fatty acid synthase activities (Lissner *et al.*, 1987).

However, until now, no study has been done to investigate the potential of *S. crispus* leaves in the treatment of obesity. The local consumption of *S. crispus* as tea and claims that it can control body weight combined with its biochemical properties and in vivo effects has sparked the initiation of the investigation on health-promoting potentials of *S. crispus* as an anti-obesity agent. Thus, we evaluated the anti-obesity effect of 1% (w/w) *S. crispus* leaves extract (SCE) on diet-induced obese rats in the current study.

Materials and Methods

Animals

54 male adult male Sprague-Dawley rats aged 3 months weighing 350-450 g were purchased from Perniagaan Usaha Cahaya (Malaysia). These animals were of the same age and delivered from the supplier in the same batch. Upon received, rats were acclimatized for 7 days by giving ad libitum normal rat chow and water in the animal house. During all experiments, rats were housed in a temperature-controlled room (22±2^{o}C) with a 12-hour light/dark cycle. Protocols for animal experiments were approved by the Animal Care and Use Committee (ACUC) of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

Diet composition

The ingredients of normal rat chow pellets obtained from Miba Mansura (Malaysia) were: palm kernel meal (26%), soybean oil (4%), cornstarch (46%), mineral mixture (3.5%), vitamins mixture (1%), L-cystine (0.18%), choline bitartrate (0.25%). The diet was formulated from the AIN-93M diet for maintenance of rodents when casein is used as the protein source (18) and modified. HF diet was prepared from a mixture of 50% normal rat chow pellet (Miba Mansura Trading, Malaysia), 20% of full-cream milk powder (Nespray brand from Nestlé), 6% sugar and 24% of corn oil (Mazola brand). The diet contained 40.5% carbohydrate, 16.1% protein, 31.1% fat, 2.5% fiber and 5.1% ash. Proximate composition was expressed as % weight of diet and was calculated by the researchers.

Preparation of Strobilanthes crispus *extract (SCE)*

Strobilanthes crispus ZII 109 (L.) Bremek (family Acanthaceae) leaves were taken from Botanical Garden of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The collected leaves were cleaned with tap water and immediately dried on an air oven (Memmert, Schwabach, Germany) at 40°C overnight. Dried leaves were grinded to fine powder using an electric blender (Philips, Malaysia). Powdered leaves were sealed in plastic bags and kept at 4˚C before use. To obtain crude extract, powdered leaves were soaked and stirred continuously with chloroform-methanol (5:3) for 12 hours. Crude extract within solvents was poured onto a round flask before being connected to a rotary evaporater (Rotavapor® R-210/R-215, Büchi, Switzerland) to remove the solvents. Solvent-free crude extracts were stored at -20°C before further use.

Induction of obesity

High-Fat Diet (HFD) treatment was started at Week 1 after rats were randomly divided into two groups: Normal diet group (n=12) and HFD group (n=42). Food and water for both groups were given ad libitum. At Week 14, the body weight and body weight gain of both groups were statistically analyzed. Rats from HFD group were considered as diet-induced obese (DIO) if their bodyweight is 30% more than Normal diet group. Six rats from Normal diet group and 6 DIO rats were sacrificed to obtain the Baseline data.

Experimental design

Six rats from Normal diet group and 12 DIO rats from the previous experiment were used. Rats were assigned to 3 groups of: a) Negative control, normal rats (n=6), b) Positive control, DIO rats (n=6) and c) Treatment group, DIO rat treated with SCE (n=6). However, towards the end of the experiment, positive

control group only consisted of 5 rats as 1 had died because of mishandling. All groups were given normal rat chow. Negative control and Positive control group were given tap water while Treatment group were given SCE in drinking water. At Week 28, rats were sacrificed to obtain the Final data.

Food intake, body and organ weight

The amount of food taken by rats in each cage and their individual bodyweight were recorded once a week using an electrical balance (AND, HR-200, Singapore). Adipose tissue was dissected from adipose depots consisted of the left and right inguinal, retroperitoneal adipose depots, and mesenteric adipose depot. Liver, left and right kidneys, heart and pancreas were also dissected. All of these organs and tissues were weighed.

Biochemical parameters

Blood withdrawn from the cardiac puncture of each rat were collected into separate EDTA tubes. Blood glucose level were determined using a glucose monitoring system (True Track Smart System Meter®) purchased from Home Diagnostics Inc., USA. The remaining tubes were centrifuged at 3000 RPM, 4°C for 15 minutes to obtain the plasma. To obtain the total cholesterol, triglycerides, HDL and LDL levels, 50 µl of plasma was analyzed using an automated clinical chemistry analyzer (Hitachi 902, Roche Diagnostics). Leptin level was analyzed using a Rat Leptin Assay Kit (L) (IBL Co. Ltd., Japan - Code No. 27295) purchased from All Eights Sdn. Bhd. (Malaysia). The IBL's Rat Leptin Enzyme Immunoassay Kit (L) is a complete kit for the quantitative determination of Rat Leptin in serum, EDTA-plasma and supernatant of cell culture media. Plasma glycerol level was analyzed using a Glycerol Colorimetric kit purchased from Randox Laboratories (Malaysia).

Liver histology analysis

Livers were cut on the same lobe for each sample to obtain a small sample and fixed in 10% formalin for further use. Prior to staining, liver tissues were dehydrated in a tissue processor machine (Leica TP1020, Germany) and blocked using paraffin wax. Blocks were kept at 0ºC for 3 hours, before tissues were trimmed at 4µm per layer using a microtome (Letiz Wetzlar 1512, Germany) to 4 µm and pasted on slides and dried on a hot plate at 50-55ºC for 30 min and then kept at 37ºC. Routine Mayer's Haematoxylin and Eosin (H & E) staining method was used. After perfectly dried from xylene (Ajax Chemicals, Auburn, Australia), the slides were mounted with cover slips and a drop of DPX mountant for microscopy. The slides were then dried at room temperature for two days and tested under a light microscope (Olympus CK2, Japan).

Statistical analysis

Data was analyzed using SPSS window program version 22.0. Comparison for each of the clinical variables was done using Independent T-test for comparisons of 2 groups and 1-way ANOVA for comparisons using more than 2 groups. Significant interactions were analyzed with Tukey post-hoc test and Tukey corrected p-values <0.05 were considered significant.

Results and Discussion

High-fat diet induced obesity in rats

During induction of obesity, although the amount of food intake in rats fed high-fat diet is similar to the rats fed normal rat chow diet, their caloric intake was significantly different ($p<0.05$). Mean energy intake per day per rat during the whole 14 weeks of diet treatment differed significantly ($p<0.05$) between the Normal group and HF diet group indicating that HFD group did not decrease their food intake despite the high calories provided by the diet given. The higher energy intake per day in HFD group also resulted in higher bodyweights. At Week 14, 18 rats in the HFD group became obese (O) with significantly ($p<0.05$) higher mean weight gain than Normal rats. Feed efficiency was increased in the HFD group although not significant (Table 1).

High-fat diets often promote greater caloric intake and/or weight gain than lower fat diets, as revealed by multiple research paradigms including epidemiological studies, experimental manipulation of dietary fat content in human subjects, and animal models of diet-induced overeating (Warwick and Schiffman, 1992; Warwick and McGuire, 2000). The same case applied to this experiment. It was found that HF group consumed almost double the energy consumed by the Normal group. This result is not in line with the traditional belief that fat is the macronutrient with the strongest satiety property. Presumably, foods with high satiation or satiety value should aid in controlling energy intake, whereas items with low values should provide a weaker barrier to consumption. However, more recent literature provides compelling evidence that fat is actually the least satiating of the macronutrients (Blundell and Mac Diarmid, 1997; Tso and Liu, 2004).

Normal diet group during induction of obesity consumed normal rat chow, where significantly less bodyweight gain was found compared to HFD group

Table 1. Bodyweight, food intake, organ weights and lipolysis rate

	Baseline Data, Week 0 (n=54)	After Induction of Obesity Week 14		After Treatment with SCE Week 28		
		Normal Diet Group (n=12)	HFD Group (n=42)	Negative Control Group (n=6)	Positive Control Group (n=6)	Treatment Group (n=6)
Bodyweight (g)	391.17 ± 33.33	488.67 ± 31.85	535.67 ± 33.1*	512.5 ± 8.76^{a}	577.8 ± 34.32^{b}	547.67 ± 35.18^{ab}
Bodyweight gain / week (g/week)	NT	6.03 ± 5.51	9.64 ± 7.03	3.53 ± 6.07^{a}	2.63 ± 2.71^{a}	0.48 ± 1.05^{b}
Food intake (g/rat/day)	NT	27.3 ± 4.72	25.71 ± 3.22	26.65 ± 1.25	25.23 ± 1.35	25.15 ± 1.22
Feed efficiency (Weight gain (g) / caloric intake X 10^2)	NT	6.32 ± 1.58	7.22 ± 1.52	7.11 ± 1.72^{a}	7.27 ± 1.39^{a}	4.62 ± 1.17^{b}
Adipose tissue (g)	NT	13.65 ± 2.92	29.23 ± 8.46*	17.81 ± 2.13^{a}	27.87 ± 10.53^{b}	15.25 ± 5.67^{a}
Liver (g)	NT	12.08 ± 1.67	14.68 ± 0.7*	12.52 ± 0.75^{a}	13.42 ± 1.02^{b}	12.07 ± 0.4^{a}
Kidney (g)	NT	3.08 ±1.67	3.2 ± 0.2	3.19 ± 0.34	3.57 ± 0.12	3.26 ± 0.2
Heart (g)	NT	1.52 ± 0.84	1.53 ± 0.21	1.42 ± 0.15	1.53 ± 0.17	1.58 ± 0.18
Pancreas (g)	NT	1.18 ± 0.28	1.12 ± 0.33	0.97 ± 0.36	1 ± 0.05	1.13 ± 0.14
Lipolysis rate [Glycerol (µmol/L)/Adipose tissue (g)]	NT	9.20 ± 3.56	3.35 ± 1.21*	8.37 ± 1.17^{a}	6.11 ± 2.18^{b}	10.67 ± 3.58^{a}

NT - Data were not taken

All data are means ±SD. Significant differences in Diet Effects Data were statistically analyzed using t-test. Significance were valued at $p<0.05$ and marked with *. Significant differences in Treatment Effects Data were statistically analyzed using one-way ANOVA. Significance were valued at $p<0.05$ and marked with different alphabet.

which consumed high-fat diet (Table 1). Consumption of a diet high in fat is often associated with greater caloric intake and weight gain than occurs on a low fat diet. This has been revealed in epidemiological studies, via experimental manipulation of dietary fat content using human subjects, and in animal models of dietary-induced overeating (Warwick and Schiffman,1992; Warwick and McGuire, 2000). Higher feed efficiency was also found in HFD group compared to Normal Diet Groups. Obese rats utilized the most of its energy intake to increase weight gain as been found by other similar studies (Levin *et al,* 1997; Yang *et al*, 2001; Dourmashkin *et al,* 2006; Lee *et al,* 2006).

Except for the low HDL level, Obese rats did not show the attributes usually found in rats given high-energy diet such as higher cholesterol, LDL and cholesterol level compared to Normal rats (Moreno *et al*, 2006; Ono *et al,* 2006). Two earlier studies (Gao *et al,* 2002; Kusunoki *et al*, 2005) have shown that the level of triglycerides in rats given HF diet was far greater than the level of triglycerides found in this experiment. The high-fat diet given to HFD group did not induce high level of triglycerides in the blood. Plasma HDL level in found in this experiment is similar to a study done by Kusunoki and colleagues (2005) which is 1.31 mmol/l in rats fed normal rat chow diet and 1.33 mmol/l in rats fed high-fat diet.

Among all the organs weights measured, the only significant differences ($p<0.05$) found between the two groups were of adipose tissue and liver weight. Adipose tissue weight of HFD group was found to be 114% higher while liver weight was 16% higher than Normal group (Table 1). Moreover, from observation, liver of HFD group was seen to be milky in color compared to the dark red colour of Normal diet group. Further histology analysis confirmed high amount of fat had accumulated in the liver of HFD group based on the amount of white circles (lipid droplets) in the liver cells (Figure 1). Animal studies done by many others also confirmed that diet-induced obese rats given high-energy diet developed fatty liver based on the milky color of the liver, by its' higher weight and by H&E staining method compared to Normal rats given normal rat chow diet (Takeda *et al*, 2001; Morimoto *et al,* 2005; Moreno *et al,* 2006).

Effect of SCE on food intake, feed efficiency, body weight of rats

During treatment with SCE, the diet of high-fat diet induced obese rats was switched to normal rat chow. Despite the changes in diet, food intake remained constant in all groups. Other studies have shown that over the course of a few days, the weight of the food consumed was more constant than the calories eaten (Poppitt and Prentice, 1996; Rolls and Bell, 2000; Yao and Roberts, 2001; Kral and Rolls, 2004).

Mean food intake per day in all 14 weeks of treatment was found to be not significant between the groups. As all of the rats consumed the same diet, this means that the energy intake between the groups were also similar. However, bodyweight gain per week of Treatment Group was found to be significantly lower than both Negative and Positive Control Groups (Table 1). This leads to significantly lower feed efficiency in the Treatment Group. Although lower feed efficiency in Treatment Group should lead to bodyweight reduction, no statistically

Table 2. Plasma glucose, leptin and lipid profile

	Baseline Data, Week 0 (n=54)	After Induction of Obesity Week 14		After Treatment with SCE Week 28		
		Normal Diet Group (n=12)	HFD Group (n=42)	Negative Control Group (n=6)	Positive Control Group (n=6)	Treatment Group (n=6)
Fasting blood glucose (mmol/L)	NT	5.48 ± 1.6	6.15 ± 0.64	11.57 ± 1.64	15.82 ± 6.74	10.63 ± 2.22
Plasma leptin (ng/ml)	NT	6.29 ± 1.01	15.87 ± 3.82*	7.37 ± 1.21[a]	13.69 ± 3.51[b]	6.81 ± 1.71[a]
Plasma glycerol (µmol/L)	NT	101.24 ± 17.21	121.14 ± 41.45	147.7 ± 13.71	155.72 ± 39.5	147.1 ± 7.76
Plasma HDL (mmol/L)	NT	1.33 ± 0.23	1.17 ± 0.05	1.1 ± 0.21	1.32 ± 0.11	1.11 ± 0.08
Plasma LDL (mmol/L)	NT	0.3 ± 0.01	0.2 ± 0.02	0.32 ± 0.05	0.36 ± 0.14	0.34 ± 0.14
Plasma Triglycerides (mmol/L)	NT	0.78 ± 0.41	0.68 ± 0.16	0.43 ± 0.04	0.66 ± 0.4	0.42 ± 0.02
Plasma Cholesterol (mmol/L)	NT	1.8 ±0.26	1.6 ± 0.09	1.58 ± 0.24	1.8 ± 0.04	1.64 ± 0.38

NT - Data were not taken

All data are means ±SD. Significant differences in Diet Effects Data were statistically analyzed using t-test. Significance were valued at $p<0.05$ and marked with *. Significant differences in Treatment Effects Data were statistically analyzed using one-way ANOVA. Significance were valued at $p<0.05$ and marked with different alphabet.

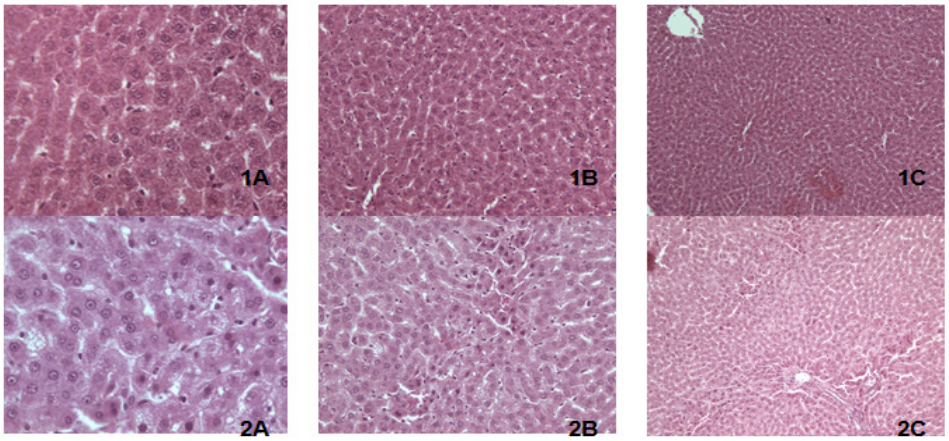

Figure 1. Histology analysis of liver after induction of obesity

The liver of rats fed the standard rat chow diet is normal and shows very mild occurrence hepatic steosis (1A, 1B, 1C). The livers of the Obese rats fed HF diet showed pronounced hepatic steosis (2A, 2B, 2C). Note: Pictures 1A and 2A - magnified 40X, 1B and 2B - magnified 20X, 1C and 2C –magnified 10X.

significant difference in the bodyweight of all groups. Even though there was no significant difference ($p>0.05$) between the total body weight gain in all of the groups, the value at Week 28 increased in the order of Negative Control Group<Treatment Group<Positive Control Group (Table 1).

Effect of SCE on leptin level, lipid and glucose profile

Treatment with SCE had significantly ($p<0.05$) lowered the plasma leptin level. No significant ($p>0.05$) differences was found in the plasma glucose and lipid level of rats in all groups (Table 2). However, it is worthy of note that the mean plasma TG, cholesterol and blood glucose level in Positive Control group was found to be the highest of all groups.

Results from the treatment shown that SCE had no significant effect on plasma lipid profile even though some mild effect on TG and cholesterol lowering were noted (Table 2). The TG level found in this experiment is similar to studies done by Kwon with colleagues (2003) and Nakayama with colleagues (2007). Nakayama (2007) found that control rats given high-fat diet had a TG level of 0.56mmol/l while rats given high-fat diet with Chinese herbal medicines had TG level of 0.34mmol/l. In another study, Kwon (2003) found that control rats given high-fat diet had a TG level of 0.66mmol/l compared to rats given high-fat diet with 5% *Dioscorea nipponica* methanol extract had a TG level of 0.46mmol/l. Thus SCE had better effect on TG lowering than *Dioscorea nipponica* methanol extract. We can also conclude that normal rat chow had did not change the lipid profile of Positive Control group even though their diet was switched.

The effect of SCE on plasma glucose level,

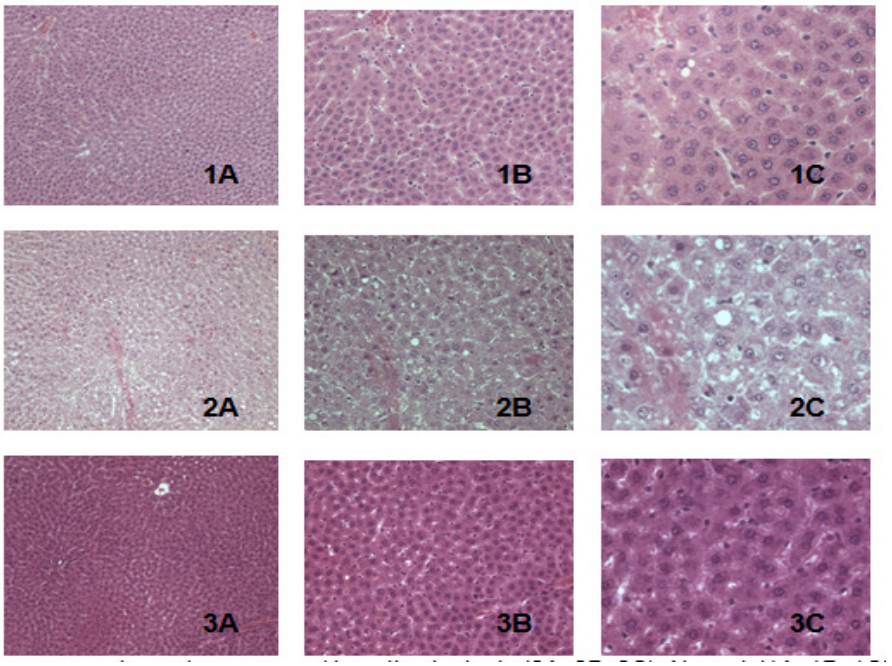

Figure 2. Histology analysis of liver after treatment with SCE

Note: The liver of Obese group showed pronounced hepatic steatosis (2A, 2B, 2C). Normal (1A, 1B, 1C) and OT (3A, 3B, 3C) groups showed mild occurrence of hepatic steatosis. Pictures were magnified (A - 10X, B – 20X, C – 40X).

although not significant, it was definitely lower than Positive Control Group. Similar results was found by Gao and colleagues (2002), where diet-induced obese Sprague-Dawley rats were maintained on a high-fat diet for 6 months followed by a low-fat diet for 1 month. They found that even though high-fat diet was switched to low-fat diet, rats failed to lower their glucose level. In this experiment, glucose level in Treatment group was decreased by 32.8% compared to Positive Control group which may suggest that SCE may also have anti-diabetes properties.

Effect of SCE on lipolysis, organ weights and liver morphology

Treatment with SCE has also lowered ($p<0.05$) adipose tissue and liver weights of Treatment Group. When calculated, the significantly lower adipose tissue weight is an indicator of significantly ($p<0.05$) higher lipolysis rate in Treatment Group (Table 1). The lipolysis rate increased in the order of Positive Control Group<Negative Control Group<Treatment group (Table 1). No significant differences ($p >0.05$) were found in the kidney, heart and pancreas weight of all groups (Table 1). Adipose tissue weight was highest in Positive Control group, followed by Negative Control and Treatment group. Liver weight also followed the same pattern (Table 1).

Occurrence of hepatic steatosis had decreased (Figure 2) in Treatment group compared to Positive Control Group. Livers of Treatment group show similar degree of mild hepatic steatosis as Negative

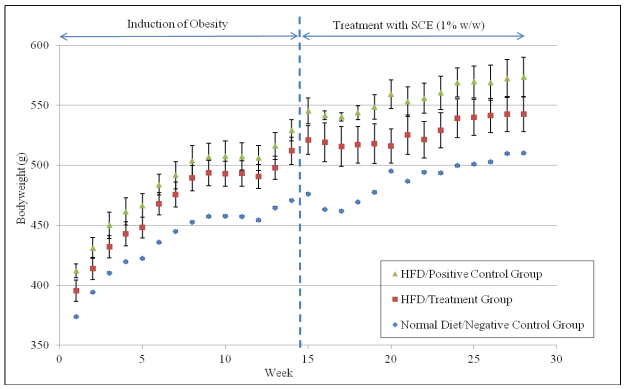

Figure 3. Bodyweight changes before and after treatment with SCE

Control group. At Week 28, Positive Control group, however, had developed a severe case of hepatic steatosis (Figure 2), more severe than HFD group at Week 14 (Figure 1). Histology analysis on the liver had support the suggestion derived from adipose tissue and liver weights data. The livers of Treatment group had been found to improve their color appearances and have a lower degree of hepatic steatosis compared to Positive group (Figure 3.0).

Weight loss of adipose tissue and liver after/during treatment with suspected anti-obese plants or herbs or compounds had been associated with body weight loss and an improved state of obesity. Raspberry ketone, a major aromatic compound of red raspberry (Marimoto *et al.,* 2005), Diocorea nipponica methanol extract (Kwon *et al.*, 2003), Arachis hypogaea nutshell extract (Moreno et al,

2006), Chinese herbal medicines: Inchinko-to, Bofu-tsusho-san and dai-saiko-to (Nakayama *et al.*, 2007) and *Nelumbo nucifera* leaves extract (Ono *et al*, 2006) were also found to lower adipose tissue and/or liver weights after/when given HF diet.

Conclusions

In summary, the formulation that we had developed had successfully induced obesity in Sprague-Dawley rats. The diet-induced obese rats had all the physical and biochemical attributes of obesity that is similar to the human obesity. The state of obesity in HFD group remained persistent even though diet was switched to normal rat chow diet in the Positive Control group. Treatment with SCE had improved their state of obesity with significantly lower bodyweight gain, leptin level, adipose tissue and liver weight, higher lipolysis rate, better liver color and improved state of hepatic steatosis even though there was no significant effect on bodyweight loss. Profoundly, the positive effect of *S. crispus* in assisting adipose tissue weight loss can be due to the enhanced rate of adipose tissue lipolysis. Thus, we can conclude that 1% (w/w) *S. crispus* extract may, in part, play a role in adipose tissue weight loss of DIO rats but more studies needs to be done in determining the dose response effects of SCE to verify the best dose. It is also necessary to investigate the effect of SCE on obesity related gene expression to determine the specific mechanisms involved in the lipolysis effect of SCE.

Acknowledgements

We thank the staff at the Universiti Putra Malaysia animal house for the maintenance of our rat colony and assistance with the diet studies and Radhiah Shukri for advice in formulating the diets. We also thank Siti Muskinah, Ghanya Al-Naqeeb and Catherine Baun Dugong from Faculty of Medicine and Health Sciences for their technical assistance. This work was supported by a grant from the Ministry of Science, Technology and Innovation, Government of Malaysia (IBS 54385).

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