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For Review

Essential oil from *Citrus aurantifolia* prevents ketotifen-induced weight-gain in mice

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Obesity is a major health problem world-wide. Medical intervention is often needed to tackle this problem, and accordingly the need for developing more effective, safer and cheaper weight reducing drugs has become paramount in recent years. In the present study, the effects of lime (*Citrus aurantifolia*) essential oils in reducing body weight, individually and in co-administration with ketotifen, an antihistaminic drug that causes weight-gain, has been investigated using mice model. During the 45 days experimental period, the mice that received ketotifen demonstrated an enhancement both in the amount of food intake and body weight compared to the control group. Groups treated with lime essential oil displayed reduction in body weight and food consumption in mice, possibly through promoting anorexia which might have played a role in weight loss. Interestingly, co-administration of the lime essential oil and ketotifen caused significant suppression in gaining weight, as well as decreased body weights of mice. The data obtained in this study suggested that lime essential oil plays an important role in weight loss and could be useful in treatment of drug-induced obesity and related diseases. The GC-MS analysis of the essential oils of *C. aurantifolia* was also performed and approximately 22 main components, with limonene (28.27%) being the principal one, were identified and quantified.

***Keywords: Citrus aurantifolia*; Rutaceae; ketotifen; weight change; mice; GC-MS**

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INTRODUCTION

Modern civilisation is faced with an epidemic of overweight and obesity which now affects nearly one-third of the world's population, and it is continuing to rise (Prentice, 2006). The number of overweight children has doubled in the last couple of decades (Yackobovitch-Gavan et al., 2008). Obesity and overweight pose major risks for serious diet-related chronic diseases, including type-2 diabetes, cardiovascular disease, hypertension, stroke, musculoskeletal disorders like osteoarthritis, and certain forms of cancer (Nasr and Drury, 2008; Saito et al., 2010). While the causes of obesity are quite varied and complex (Harris et al., 2002), there are a number of medicines in use today which cause weight-gain and obesity (Pouzet et al., 2003; Arjona et al., 2004; Malone, 2005; Levine and Saltzman, 2006; Laimer et al., 2006; Gobshtis et al., 2007; Sato et al., 2007). Antihistamines are among the most common medicines in use today in over-the-counter (OTC). As side effects, most antihistamines, e.g. ketotifen, not only sedate and slow down metabolism, but also stimulate appetite resulting in weight-gain (Rossner , 2000; Poyurovsky et al., 2005; Couluris et al., 2008; Yin et al., 2008). Obesity, induced by conventional medicines or not, has become a global problem, and medical intervention is often needed to tackle this problem. Accordingly, the need for developing more effective, safer and cheaper weight reducing drugs has become paramount in recent years.

Citrus aurantiifolia (Christm.) Swingle (family: Rutaceae), commonly known as 'lime', cultivated extensively in tropical and subtropical countries mainly for its use as a food item or food additive, is also medicinally best known throughout the world as a remedy that relieves fevers, sore throat, coughs, common cold and indigestion (Fukumoto et al., 2006; Dr Duke's

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Phytochemical and Ethnobotanical Databases, 2010; GRIN Taxonomy Database, 2010). As part of our continuing phytochemical and pharmacological studies on *Citrus* species (Sarker et al., 2008; Miah et al., 2010), we now report on the assessment of the effects of lime (*C. aurantifolia*) essential oils in preventing ketotifen-induced weight-gain in mice.

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MATERIALS AND METHODS

Materials. Ketotifen (Hakim Pharmaceutical Company, Iran), normal saline (NaCl) (Shahid Ghazi, Tabriz-Iran), di-methyl-sulfoxide (DMSO) (Merck, Darmstadt, Germany) and lime oil (extracted from fruits of *Citrus aurantifolia* by Barij-Essence Company, Iran) were used in this study.

GC-MS analysis. Chemical profile of lime essential oils varies from variety to variety of *Citrus aurantifolia*; hence it was necessary to assess the essential oil further using the GC-MS (Fukumoto et al., 2006; Nguyen et al., 2009; Razavi et al., 2009). The lime essential oil was analyzed by the GC-MS using a Shimadzu GC-MS-QP 5050A gas chromatograph fitted with a DB5 (polydimethylsiloxane, 60 m × 0.25 mm i.d.) capillary column. Carrier gas, helium with a flow rate of 0.9 mL/min; column temperature, 3 min in 60°C, 60-270 °C at 3 °C/min; injector temperature, 250 °C detector temperature, 280 °C, Volume injected, 1 µL of lemon essential oil in n-hexane (2%); Split ratio, 1:44. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature; 280 °C; quadrupole 100 °C, Solvent delay 10 min, scan speed 2000 amu/s and scan range 30-600 amu, EV voltage 3000 volts.

Identification of compounds. The identification of compounds was based on direct comparison of the retention times and mass spectral data with those for the standards and by computer matching with the Wiley 229, Nist 107, Nist 21 Library, as well as by comparison

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of the fragmentation patterns of the mass spectra with those reported in the literature (Massada, 1976; Paresh et al., 1998; Adams, 2004; Ramesh et al., 2004).

Animals. Male mice weighing 25-30g were used in this survey. Animals were maintained under standard environmental conditions and had free access to feed and water *ad libitum*. Experiments on animals were performed strictly in accordance with the guidelines provided by the Institutional Animal Ethics Committee, internationally accepted principles and the national laws concerning the care and the use of laboratory animals. The animals were housed in metal cages with wire-mesh tops under controlled environmental conditions (temperature 21±1 ^{o}C, with a light/dark cycle of 12 h). The mice were allowed free access to 250 mg of concentrated animal food and water every day.

Study protocols. In this study 56 male mice (25-30 g) were selected and randomly assigned to seven groups (n=8). Seven groups of animals received during 45 consecutive days: normal saline as a control group (0.1 mL/mice, sc.), DMSO as a control group (0.02 ml/mice, sc.), one group of ketotifen (32 mg/kg, sc.) dissolved in 0.1 mL of normal saline, three groups with different doses of lime essential oil (125, 250, 500 mg/kg, sc.) dissolved in 0.02 mL of DMSO and one group with mixture of ketotifen and lemon essential oil (32 mg/kg, sc. and 125 mg/kg, sc., respectively) separately dissolved in normal saline and DMSO as above mentioned.

Food intake and body weight changes. The amount of food intake was measured by weighing the jars containing food daily and body weights of the mice were also evaluated every two days and were recorded.

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Statistical analysis. All data are expressed as mean ± S.D. Differences between treatments groups were assessed by T-test and StatView software. A probability level of $P < 0.05$ was taken to be statistically significant in the analyses.

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RESULTS AND DISCUSSION

The GC-MS analysis of the essential oil of *C. aurantifolia* led to the identification and quantification of approximately 22 main compounds, which accounted for 88.85% of the total components present. Limonene (28.27%) was the principal component followed by α-terpineol (19.61%), *p*-cymene (8.6%), and β-pinene (5.7%). Other components of the essential oil comprised low portions of it, as is indicated in Table 1. Although the identified components of the essential oil of *C. aurantifolia* were similar to prior published data (Chisholm, 2003; Nickavar and Mojab, 2003), there were minor differences probably because of variations in growing conditions (Yannovits-Argiriadis, 1992).

The amounts of food intake data are presented in Figure 1A-C. There was a trend of increase in the amount of food consumption during the 45 days in the group received ketotifen (178 g) compared to the control group of normal saline (161 g). There was a significant difference ($p < 0.03$) between the two groups. Notably less food intake (about 110 g) was observed with the groups that received lime essential oil with respect to the control group of DMSO (145 g) ($p < 0.001$) (Figure 1B). The graph in Figure 1C indicates that there was a clear gap in the amount of food intake between the groups which received ketotifen or essential oil independently, whilst when they were administrated together, there was a significant modification in the amount of food intake in mice (132 g) ($p < 0.001$).

Scatter diagrams were used to determine and compare the weight changes of mice in different groups, during the 45 days of the study (Figure 2). Administration of 32 mg/kg ketotifen in

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the mice resulted in an increase in body weights from 32 g to 62 g. There was a noticeable weight differences between control group of normal saline and ketotifen. Previous studies support the idea of gaining weight in usage of antihistamines (Grant et al., 1990).

The effect of administration of lime essential oil in three doses of 125, 250 and 500 mg/kg was assessed. As exhibited in Figure 2B, mice receiving lime essential oil showed a decrease in body weight (33 g to 20 g) and total food intake over the 45 days study period. Body weights and the amount of food intake in essential oil treated groups were substantially less than the control group of DMSO that showed almost invariable weights during 45 days of the examination. Importantly, mortality, not observed in any other groups, was recorded in the 500 mg/kg lime essential oil treated group. Incident of mortality showed sensitivity to the amount of dose they received, greater doses caused more deaths. Although, low doses of the lime essential oil appeared to be well tolerated and safe (Ceccarelli et al., 2004; Campbell et al., 2006), these experiments revealed a necessity in adjusting dose of lime essential oil cautiously in administration, particularly in human beings (Michaelakis et al., 2009).

Body weight changes were studied with co-administration of the ketotifen and lime essential oil. Ketotifen caused weight gain in the treated group, and weight loss was observed in the groups received low doses of the lime essential oil together with kitotifen. The result indicated that administration of lime essential oil along with ketotifen not only caused significant suppression in gaining weight, but also decreased body weights of the mice from 33 g to 23 g (Figure 1C). No adverse reactions were reported during the period.

On the whole, the mice received ketotifen there was a tendency towards weight gain as well as the amount of food they consumed, whereas mice of the control group neither showed

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changes in body weights nor in food consumption. It is to be noted that, the statistical analysis did not show much difference in weights of the mice during the initial days of the experiment. Weight gain and increased food intake was observed after several days of receiving ketotifen. This is probably due to the fact that, ketotifen injections stimulated food intake after several days of receiving it, primarily by increasing appetitive feeding behaviour and the number of meals. The findings agree with many previous reports (Grant et al., 1990).

In contrast, results of lime essential oil demonstrated subtle weight loss with decreased food intake, while the control group of normal saline showed little or no changes. It is likely that receiving lime essential oil suppresses appetite of the mice that not only brings about reduce in consumption of the food but also causes weight loss.

The evidences implicated that ketotifen had an influence in mealtime hunger and meal initiation and body weights of the mice were closely related to daily food consumption. Nevertheless, when mice received lime essential oil along with ketotifen, lime essential oil moderates compensatory changes in response to body-weight alterations and induces weight loss. The weight loss appeared to be mediated through suppression in appetite. Accordingly, these data suggest that lime essential oil could play an important role in weight loss and could be useful in the treatment of obesity and related diseases, as well as to prevent weigh-gain side effects of a number of drugs, e.g. ketotifen.

In summary, the findings of the present study suggest that lime essential oil can be an excellent candidate for treatment of drug-induced obesity, since it affects food intake as well as a diverse array of processes involved in energy expenditure and fuel utilization, all of which suppress weight gain.

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REFERENCES

Adams RP. 2004. Identification of Essential Oil Component by Gas chromatography/ Quadrupole Mass spectroscopy. Allured Publishing Corporation, Illinois, U.S.A.

Arjona AA, Zhang SX, Adamson B, Wurtman RJ. 2004. An animal model of antipsychotic-induced weight gain. *Behavioural Brain Research* **152**:121-127.

Campbell JI, Mortensen A, Molgaard P. 2006. Tissue lipid lowering-effect of a traditional Nigerian anti-diabetic infusion of Rauwolfia vomitoria foilage and *Citrus aurantium* fruit. *Journal of Ethnopharmacology* **104**:379-86.

Ceccarelli I, Lariviere WR, Fiorenzani P, Sacerdote P, Aloisi AM. 2004. Effects of long-term exposure of lemon essential oil odor on behavioral, hormonal and neuronal parameters in male and female rats. *Brain Research* **1001**:78-86.

Chisholm MG. 2003. Characterization of aroma volatiles in key lime essential oils (*Citrus aurantifolia Swingle*). *Flavour and Fragrance Journal* **18**:106-115.

Couluris M, Mayer JL, Freyer DR, Sandler E, Xu P, Krischer JP. 2008. The effect of cyproheptadine hydrochloride (periactin) and megestrol acetate (megace) on weight in children with cancer/treatment-related cachexia. *Journal of Pediatric Hematology and Oncology* **30**:791-797.

For Peer Review

Dr Duke's Phytochemical and Ethnobotanical Databases. 2010. USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network - (GRIN) [Online Database], National Germplasm Resources Laboratory, Beltsville, Maryland. Available on-line at: http://www.ars-grin.gov/cgi-bin/duke/ethnobot.pl?ethnobot.taxon=Citrus%20aurantiifolia

Fukumoto S, Sawasaki E, Okuyama S, Miyake Y, Yokogoshi H. 2006. Flavor components of monoterpenes in citrus essential oils enhance the release of monoamines from rat brain slices. *Nutritional Neuroscience* **9**:73-80.

Gobshtis N, Ben-Shabat S, Fride E. 2007. Antidepressant-induced undesirable weight gain: prevention with rimonabant without interference with behavioral effectiveness. *European Journal of Pharmacology* **554**:155-163.

Grant SM, Goa KL, Fitton A, Sorkin EM. 1990. Ketotifen. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in asthma and allergic disorders. *Drugs* **40**:412-448.

GRIN Taxonomy Database. 2010. USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Network - (GRIN)* [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland. URL: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?10683 (05 March 2010)

Harris RB, Zhou J, Mitchell T, Hebert S, Ryan DH. 2002. Rats fed only during the light period are resistant to stress-induced weight loss. *Physiology and Behavior* **76**:543-550.

Laimer M, Kramer-Reinstadler K, Rauchenzauner M, Lechner-Schoner T, Strauss R, Engl J. 2006. Effect of mirtazapine treatment on body composition and metabolism. *Journal of Clinical Psychiatry* **67**:421-424.

Levine S, Saltzman A. 2006. Lithium increases body weight of rats: relation to thymolysis. *Progress in Neuropsychopharmacology and Biological Psychiatry* **30**:155-158.

For Peer Review

Malone M. 2005. Medications associated with weight gain. *The Annals of Pharmacotherapy* **39**:2046-2055.

Massada Y. 1976. Analysis of Essential Oil by Gas Chromatography and Mass Spectrometry. John Wiley and Sons, New York, U.S.A.

Miah MN, Bachar SC, Nahar L, Rahman MS, Rashid MA, Hadiuzzaman S, Sarker SD. 2010. Composition of the volatiles of *Citrus macroptera* var. *annamensis* and evaluation of bioactivity. *Journal of Essential Oil Bearing Plants* (in press).

Michaelakis A, Papachristos D, Kimbaris A, Koliopoulos G, Giatropoulos A, Polissiou MG. 2009. *Citrus* essential oils and four enantiomeric pinenes against *Culex pipiens* (Diptera: Culicidae). *Parasitology Research* **105**:769-773.

Nasr SZ, Drury D. 2008. Appetite stimulants use in cystic fibrosis. *Pediatric Pulmonology* **43**:209-19.

Nguyen H, Campi EM, Roy-Jackson W, Patti AF. 2009. Effect of oxidative deterioration on flavour and aroma components of lemon oil. *Food Chemistry* **112**:388-393.

Nickavar B, Mojab F. 2003. Volatile constituents of the dried fruit of *Citrus aurantifolia* from Iran. *Journal of Medicinal and Aromatic Plant Sciences* **25**:400-401.

Paresh CD. 1998. Capillary column gas–liquid chromatographic separation of 5-unsaturated and saturated phytosterols. *Journal of Chromatography* **816**:177-184.

Pouzet B, Mow T, Kreilgaard M, Velschow S. 2003. Chronic treatment with antipsychotics in rats as a model for antipsychotic-induced weight gain in human. *Pharmacology Biochemistry and Behavior* **75**:133-40.

Poyurovsky M, Pashinian A, Levi A, Weizman R, Weizman A. 2005. The effect of betahistine, a histamine H1 receptor agonist/H3 antagonist, on olanzapine-induced weight gain in first-episode schizophrenia patients. *International Clinical Psychopharmacology* **20**:101-103.

For Peer Review

Prentice AM. 2006. The emerging epidemic of obesity in developing countries. *International Journal of Epidemiology* **35**:93-99.

Ramesh Yadav A, Chauhan AS, Rekha MN, Rao LJM, Ramteke RS. 2004. Flavour quality of dehydrated lime [*Citrus aurantifolia* (Christm.) Swingle]. *Food Chemistry* **85**:59-62.

Razavi SM, Nazemiyeh H, Delazar A, Asnaashari S, Hajiboland R, Sarker SD. 2009. Chemical variation of the essential oil of Prangos uloptera DC. at different stages of growth. *Natural Products Research* **14**:1-6.

Rossner S. 2000. H2 receptor blockaders and weight control. *Lakartidningen* **97**:3299.

Sato I, Arima H, Ozaki N, Ozaki N, Watanabe M, Goto M. 2007. Peripherally administered baclofen reduced food intake and body weight in db/db as well as diet-induced obese mice. *FEBS Letters* **581**:4857-4864.

Saito Y, Kita T, Mabuchi H, Matsuzaki M, Matsuzawa Y, Nakaya N. 2010. Obesity as a risk factor for coronary events in Japanese patients with hypercholesterolemia on low-dose simvastatin therapy. *Journal of Atherosclerosis and Thrombosis* **17:** in press

Sarker SD, Habibi B, Sharifi T, Asnaashari S, Nahar L, Delazar A. 2008. Effect of *Citrus aurantium* var *amara* on weight change in mice, *Oriental Pharmacy and Experimental Medicine* **8**: 222-227.

Yackobovitch-Gavan M, Nagelberg N, Demol S, Phillip M, Shalitin S. 2008. Influence of weight-loss diets with different macronutrient compositions on health-related quality of life in obese youth. *Appetite* **51**:697-703.

Yannovits-Argiriadis N. 1992. Essential oil variation in dwarf plants of *Pelavgonium sp.* capitatum, induced by a new plant growth bioregulator. *Plant Growth Regulation* **11**:132.

Yin J, Zuberi A, Gao Z, Liu D, Liu Z, Cefalu WT 2008. Effect of Shilianhua extract and its fractions on body weight of obese mice. *Metabolism* **57**:S47-S51.

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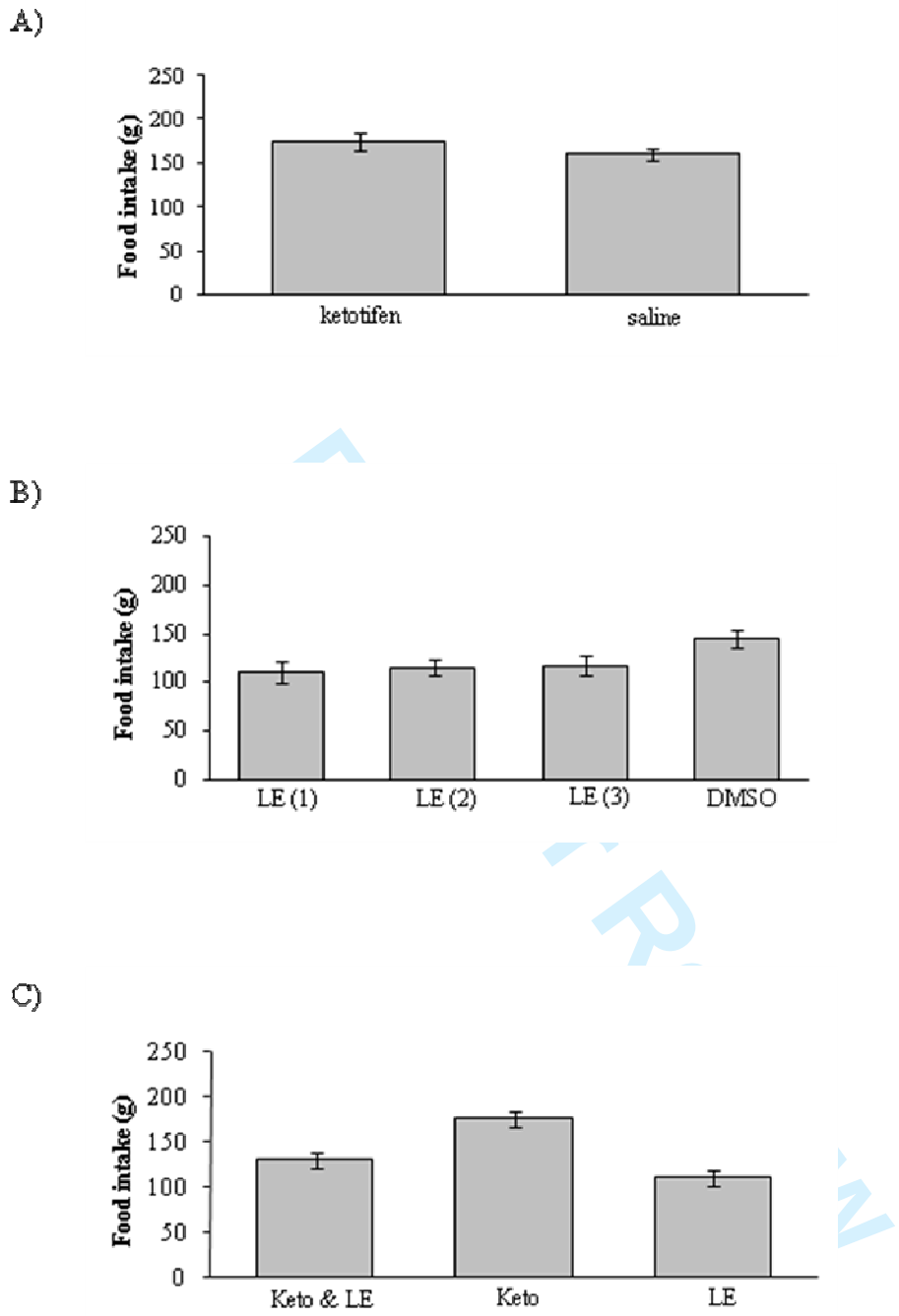

Figure 1. Effect of administrations on amount of food intake in mice; **A)** Ketotifen 32 mg/kg and control group of normal saline 0.1 mL/mouse; **B)** Lime essential oil (LE 1: 125 mg/kg, LE 2: 250 mg/kg, LE 3: 500 mg/kg) and control group of DMSO 0.02 mg/kg; **C)** Co-administration of ketotifen 32 mg/kg and lime essential oil 125 mg/kg, ketotifen 32 mg/kg, lime essential oil 125 mg/kg

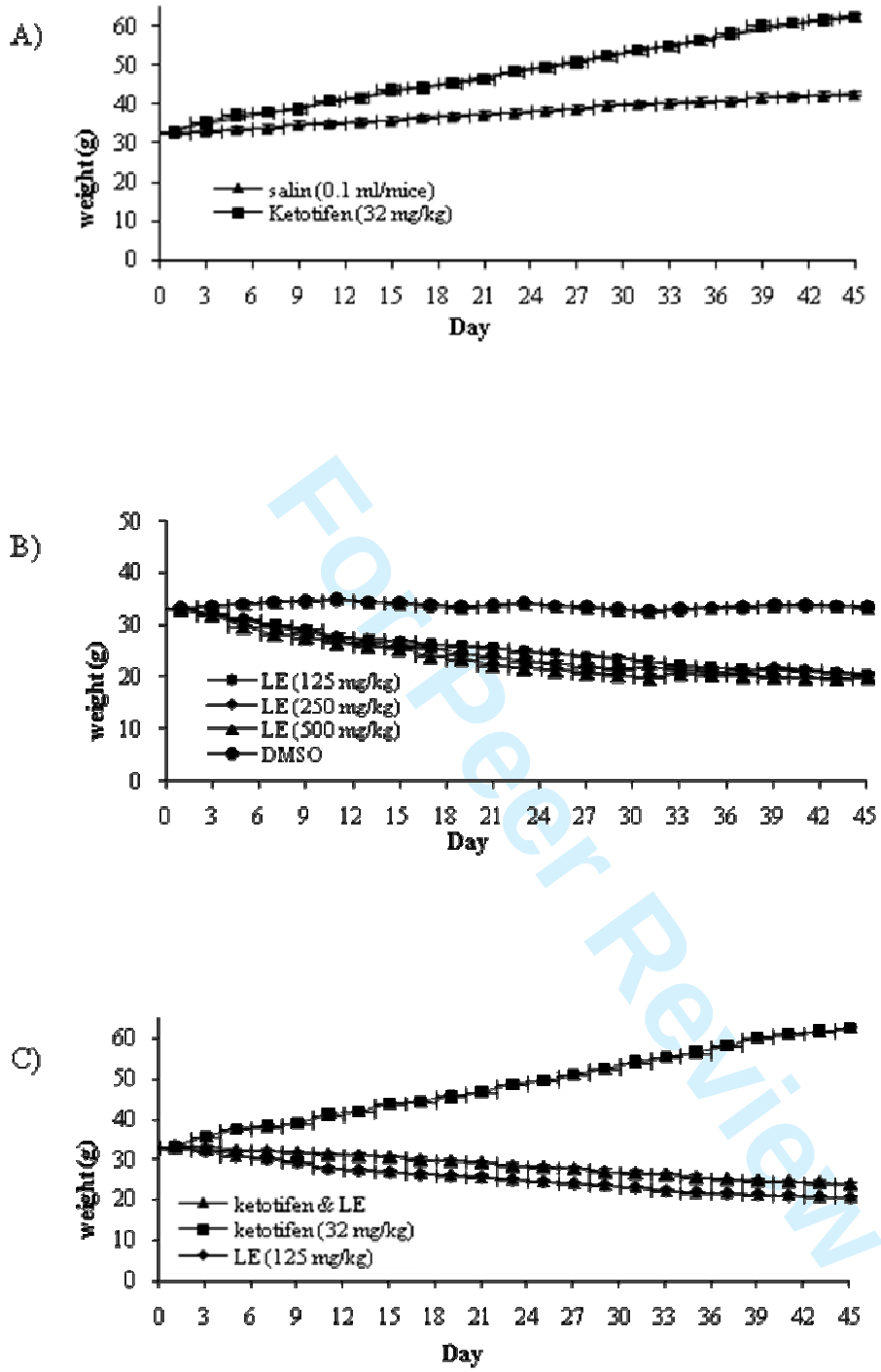

Figure 2. Effect of administrations on weight changes in mice; A) Ketotifen 32 mg/kg and control group of normal saline 0.1 mL/mouse; B) Lime essential oil (LE 1: 125 mg/kg, LE 2: 250 mg/kg, LE 3: 500 mg/kg) and control group of DMSO 0.02 mg/kg; C) Co-administration of ketotifen 32 mg/kg and lemon essential oil 125 mg/kg, ketotifen 32 mg/kg, lime essential oil 125 mg/kg.

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Table 1. Composition of the essential oil of *Citrus aurantifolia*

Peak No	Components	RI*	Composition (%)
1	α-Pinene	939	0.68
2	β-Pinene	979	5.70
3	*p*-Cymene	1025	8.58
4	D-Limoene	1029	28.27
5	*trans*-Linalool oxide	1073	0.75
6	Linalool	1097	2.39
7	Fenchol	1117	1.67
8	*cis*-Limonene oxide	1137	2.29
9	*trans*-Limonene oxide	1142	1.05
10	Pinocarveol	1142	0.58
11	β-Terpineol	1163	0.79
12	Borneol	1169	1.12
13	Terpineol-4	1177	4.76
14	*p*-Cymene-8-ol	1183	1.93
15	α-Terpineol	1189	19.61
16	Myrtenol	1196	1.17
17	*trans*-Carveol	1217	0.88
18	Neral	1238	0.79
19	Carvone	1243	0.95
20	Citral	1341	1.32
21	α-Bergamotene	1435	1.41
22	β-Bisabolene	1506	2.16

* RI is the Retention Index relative to C9–C16 *n*-alkanes on the DB-5 column

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