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Full Length Research Paper

Resting and activity-related energy expenditure: Do formerly overweight women differ from their ever-lean counterparts?

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Weight loss relapse is common in reduced-overweight and reduced-obese persons. It is unclear whether adaptations in resting metabolism and activity-related thermogenesis may result in energy-sparing, thereby contributing to weight regain. We compared resting and daily activity-related energy expenditure in formerly overweight women (maintaining weight losses of $\geq 5\%$) to normal-weight, weight-stable women matched for body mass index (BMI) and age. Reduced-overweight (RO) and normal weight (NW) women ($N = 44$) completed questionnaires for weight history, eating and physical activity behaviors. Measures included: BMI, body composition (bioelectrical impedance), resting energy expenditure and substrate oxidation, and daily activity-related energy expenditure (accelerometry). Groups were comparable for habitual energy intake, resting energy expenditure, resting fat and carbohydrate oxidation and daily activity-related thermogenesis. The RO group significantly over-estimated daily moderate intensity activity-related energy expenditure (270 min/wk) whereas the NW group did not (113 min/wk) ($P = 0.02$). Energy expenditure (resting and activity-related) was comparable in RO and NW women. With the exception of over-reporting moderate intensity physical activity, our findings suggest that formerly overweight women do not exhibit energy-sparing adaptations increasing the likelihood of weight regain.

Key words: Weight loss, weight regain, energy expenditure, fat oxidation, self-report, physical activity.

INTRODUCTION

Obesity has become a global pandemic with more than 1.5 billion persons overweight or obese, worldwide. Furthermore, it is estimated that in excess of \$60 billion, in the United States alone, is spent on weight loss efforts per year (Collins, 2013). Despite these efforts, studies show that 50 to 80% of persons who lose weight return to starting weight within 3 to 5 years following initial treatment (Weiss et al., 2007; Ross, 2009). Typically, only 20%

of overweight and obese individuals (body mass index (BMI) $\geq 27 \text{ kg m}^{-2}$) attempting weight loss, achieved at least a 10% weight loss sustained for 1 year, with most individuals maintaining only a 5% weight loss and around 35% actually gaining an additional 2 to 7 kg after a year (Wing and Phelan, 2005). These findings suggest that modest weight loss ($< 10\%$) may be more sustainable than larger weight reductions. This is noteworthy as

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sustained weight loss, even as little as 5%, is associated with reductions in blood pressure, dyslipidemia, impaired glucose tolerance (Janssen et al., 2012), anxiety and depression (McCrea et al., 2012). Thus, the key barriers to sustainable weight loss must be identified if weight loss maintenance and its associated health benefits are to be achieved among individuals who frequently relapse.

Prior research has shown that persons who are successful at maintaining weight loss have made numerous behavior changes, such that they generally weigh themselves more frequently, restrict their daily fat intake, exercise (on average) more than an hour per day and are more likely to eat breakfast, compared to weight-stable normal individuals and weight-loss relapsed obese persons (Wing and Phelan, 2005). Conversely, there is evidence that reduced-obese individuals present with metabolic and behavioral compensatory responses which oppose long term weight loss (King et al., 2007).

The metabolic compensatory responses include: a lower-than-expected resting metabolic rate, an elevated fasting resting respiratory exchange ratio (indicative of decreased metabolic flexibility) (Wyatt et al., 1999) and a reduction in resting fat oxidation rates (van Aggel-Leijssen et al., 2001). The behavioral compensatory responses (being either automatic and/or volitional) include disinhibited eating behaviors (that is, sporadic bouts of overeating) (Polivy and Herman, 1985) and a reduction in volitional and non-volitional physical activity-related energy expenditure (King et al., 2007). These findings suggest that certain individuals may have metabolic and/or behavioral profiles promoting positive energy balance and as a direct consequence, weight loss relapse.

In this study, we examined resting energy expenditure and substrate oxidation, daily activity-related energy expenditure (via accelerometry), along with self-reported habitual energy intake and physical activity behaviours in formerly overweight women (women maintaining deliberate weight losses of $\geq 5\%$) compared to ever-lean women (women who have always been normal weight), matched for BMI and age. We hypothesised that the reduced, previously overweight persons would demonstrate either metabolic or behavioral energy-sparing adaptations that may predispose them to weight-loss relapse.

METHODOLOGY

Subject recruitment

A convenience sample of 44 women, recruited from local fitness centres via notice board advertisements and two commercial weight loss enterprise electronic mail databases were included in our study. The study ran for 6 months from June to November, 2010, and participants were excluded if they experienced any of the following within the 2 months prior to enrolment: a change in total body weight of 5% or more, known metabolic disease, pregnancy or

lactation. All participants fell within a BMI range of 21 to 30, and were allocated to two groups: a reduced-overweight group (RO, N = 25) (women who experienced a $\geq 5\%$ reduction in total body weight and had successfully maintained this weight loss for at least 2 months) and a normal weight group (NW, N = 19) (a group of women with a similar BMI and age, who had never undergone deliberate weight loss). The study protocol was approved by the Research Ethics Committee of the Faculty of Health Sciences, from the University of Cape Town (HSF REC: 253/2010), and all participants gave written informed consent before visiting our laboratories.

Experimental

Questionnaire-based data obtained included: weight history, demographics, eating behavior, body shape concerns and physical activity (Global physical activity questionnaire). BMI, waist circumference, body composition, resting metabolic rate, resting respiratory quotient, reported energy and nutrient intake and actual physical activity (Actigraph GT3X) were measured.

Basic anthropometry and body composition analysis

Participants were measured for weight (BW-150, NAGATA, Tainan, Taiwan) and height (3PHTROD-WM, Detecto, Missouri, USA), with shoes removed and while wearing lightweight clothing. Waist circumference (defined here as the smallest circumference measured between the xiphoid of the sternum and the umbilicus) was determined with a standard non-elastic tape measure. Body composition was evaluated using bioelectrical impedance analysis (BIA) (Quantum II, RJL Systems, Michigan, USA). Body fat percentage (%BF) was determined using the prediction equation by Sun et al., 2003.

Self-reported physical activity using the global physical activity questionnaire (GPAQ)

The GPAQ (Trinh et al., 2009) is a self-report questionnaire that quantifies total minutes of moderate and vigorous PA in occupational (paid and non-paid work), transport and leisure time over the preceding 7 days. Only physical activity that occurred in bouts of at least 10 min duration was included. Finally, resting time (described as time spent sitting or reclining) was also quantified in minutes per day.

Three-factor eating questionnaire (TFEQ)

The 51-item TFEQ (Stunkard and Messick, 1985) measures three dimensions of human eating behavior: cognitive restraint (the tendency to restrict food intake in order to control body weight), disinhibition (a sporadic loss of dietary control in the form of increased frequency and/or volume of food intake), and hunger (a measure of whether appetite primarily drives food intake, and the extent to which the individual engages in emotional eating). Higher scores indicate greater degrees of cognitive restraint, uncontrolled or emotional eating.

Body shape questionnaire (BSQ)

The BSQ (Rosen et al., 1996) was originally developed to identify

Table 1. Basic characteristics of RO and NW subjects (mean \pm SD).

Parameter	RO (n = 25)	NW (n = 19)
Age (years)	38 \pm 11	34 \pm 10
Weight (kg)	66.4 \pm 4.7	68.4 \pm 7.5
BMI (kg/m ²)	24 \pm 2	25 \pm 2
Waist (cm)	79.1 \pm 5.2	79.9 \pm 6.1
Fat free mass (kg)	45.3 \pm 2.9	46.2 \pm 4.3
Body fat (%)	31.6 \pm 3.7	32.1 \pm 5.1
Highest adult weight (kg)	77.3 \pm 6.3	69.1 \pm 7.5*
% body weight lost	14.0 \pm 5.4 [#]	1.1 \pm 1.5*

* $P < 0.05$, [#]weight loss maintained for 2 ± 2 years; BMI: body mass index.

pre-occupations with body shape in patients suffering from eating disorders such as anorexia and bulimia nervosa and has also been used in research related to weight loss, particularly among female populations. The BSQ consists of 34 items, each yielding a score of 0 to 6 points. Points for all questions are summed and the total scores are ranked as follows: no concern with body shape = 124 to 204; mild concern with shape = 92 to 123; moderate concern with shape = 65 to 91; marked concern with shape ≤ 64 .

Food frequency questionnaire

All participants completed a previously validated, structured food frequency questionnaire (Pedro et al., 2008) (Dietary Assessment and Education Kit, Medical Research Council of South Africa, South Africa) administered by a registered dietician. Energy intake (EI) was analysed using the computer package FoodFinder™3 software application (Version 1, Medical Research Council of South Africa, South Africa). Total reported EI (kJ), total carbohydrate (CHO), protein, fat and alcohol (g and % of EI) were calculated.

Short fat questionnaire (SFQ)

The SFQ (Dobson et al., 1993) is a brief, 17-item self-administered questionnaire which provides a measure of habitual dietary fat consumption. Fifteen of the questions are worth 0 to 4 points, whereas the remaining two items can yield a maximum of 2 points each. Finally, the scores for all items are summed to generate a total out of 64 which is interpreted as follows: 0 to 17 = low fat intake; 18 to 39 = moderate fat intake; ≥ 40 = high fat intake.

Measurement of resting energy expenditure and substrate utilisation

Subjects arrived at the laboratory after a 10 to 12 h overnight fast, and then rested in the supine position for a minimum of 10 min prior to the start of the test. The ventilated hood technique was used to determine resting energy expenditure and substrate utilization through the measurement of oxygen uptake and carbon dioxide production for a total of 20 min. For each measurement, the first 5 min were discarded to ensure that subjects had reached steady state (coefficient of variance of less than 10%) (Quark RMR, Cosmed, Rome, Italy). From these measurements, resting energy expenditure (REE), using the Weir equation (Weir, 1949), respiratory

respiratory exchange ratio (RER) and fat oxidation were determined. Before the start of each testing day, the gas analyzer was calibrated with a 3 L syringe and standard gas mixtures of oxygen (26% O₂ with the balance nitrogen) and carbon dioxide (4% CO₂, 16% O₂ and the balance nitrogen) (BOC Special Gas, Afrox, Cape Town, South Africa).

Accelerometry for objective measures of physical activity

Fourteen participants in the RO group and fifteen participants in the NW group wore an accelerometer (Actigraph GT3X, Actigraph, Shalimar, FL, USA) on their hip for seven consecutive days. Participants were asked, where possible, to adhere to their "normal activities" for the duration of these seven days, despite the presence of the accelerometer. A minimum of 600 min (10 h) was required for one day of wear to be considered valid. For the current study, a minimum of 4 days of valid wear time was taken. Data from the GT3X were downloaded to a computer and were analyzed using a Matlab-designed program (Matlab, Mathworks, MA, USA). Moderate activity was differentiated from vigorous activity by cut-points according to Matthews (2005). One bout of exercise was quantified as 10 min or more, of 760 to 5998 counts per minute for moderate activity, and in excess of 5,999 counts per minute for vigorous activity. Finally, we calculated the total minutes per week of moderate, vigorous and combined bouts in minutes.

Statistical analysis

The statistical package Statistica™ 9.0 (Statsoft. Inc, Tulsa, Oklahoma, USA) was used to analyze all data. Demographic and resting metabolic data, dietary behavior and self-reported physical activity were compared between groups using either independent t-tests for normally distributed data, or the Mann-Whitney U test for data which were not normally distributed. Over-reporting or under-reporting of physical activity was estimated based on the difference in self-reported versus objectively measured minutes of moderate and vigorous physical activity (GPAQ versus accelerometry). Data are presented as means \pm standard deviations or as medians and 25th and 75th quartiles. The level for statistical significance was set at $p < 0.05$.

RESULTS

Subject characteristics

The mean age of the participants was 38 ± 11 years (19 to 58 years) and mean BMI was 24 ± 2 kg m² (21 to 30 kg m²). Twenty-three participants were normal weight (BMI = 18.5 to 24.9 kg m²), and twenty-one were overweight (BMI = 25 to 29.9 kg m²). However, no significant differences were found between the RO and NW control group for weight, waist circumference, fat-free-mass (FFM) or body fat percentage. By study design, the groups were dissimilar in terms of peak adult BMI (RO = 28.2 kg m², NW = 24.9 kg m²) and significantly different for their highest adult weight (prior to weight loss in the RO group, $P = 0.00028$) and percent body weight lost ($P < 0.00001$) (Table 1).

Table 2. Resting energy and substrate metabolism in RO and NW subjects (mean \pm SD).

Parameter	RO (n = 25)	NW (n = 19)
RMR (Kcal/day)	1229 \pm 120.2	1298.4 \pm 174.7
RMR (Kcal/kg FFM/day)	27.1 \pm 2.6	28.1 \pm 3.3
RER (VCO ₂ /VO ₂)	0.79 \pm 0.05	0.81 \pm 0.07

RMR: Resting Metabolic Rate; FFM: Fat Free Mass; RER: Respiratory Exchange Ratio.

Table 3. Self-reported energy and nutrient intake and eating behaviors in RO and NW subjects.

Parameter	RO (n = 25)	NW (n = 19)
Total EI (kcal per day)	1566 \pm 417	1493 \pm 314
Fat intake (% EI)	30 \pm 6	29 \pm 7
Carbohydrate intake (% EI)	49 \pm 8	54 \pm 8
Protein intake (% EI)	18 \pm 3	16 \pm 3*
SFQ score	20 \pm 7	21 \pm 7
TFEQ restraint factor	12 \pm 4	10 \pm 4
TFEQ disinhibition factor	7 \pm 4	7 \pm 4
TFEQ hunger factor	5 \pm 3	5 \pm 3
BSQ score	149 \pm 26	140 \pm 38

Means \pm SD; EI: Energy Intake; SFQ: Short Fat Questionnaire; TFEQ: Three Factor Eating Questionnaire; BSQ: Body Shape Questionnaire.

Physiological measures

There were no significant differences in resting metabolic rate (RMR) or resting respiratory exchange ratio (RER) between the RO women and the NW control group (Table 2).

Self-reported energy and nutrient intake

Total reported energy, fat and carbohydrate intake, measures of eating behavior or body shape concerns between groups are presented in Table 3. Reported protein intake (% of total daily energy intake) was 13% higher in the RO women compared to NW controls ($P = 0.023$).

Self-reported and measured physical activity

There were no significant differences between RO and NW women for total self-reported, total combined moderate and vigorous physical activity (minutes per week), as well as for vigorous activity alone. However, RO women

reported more minutes of moderate physical activity per week ($P < 0.05$), compared to NW women (Table 4). There were no significant differences between the groups with regard to total minutes per day spent at moderate, vigorous or moderate-to-vigorous physical activity intensity measures based on accelerometry. On average, RO women reported more moderate physical activity (mean \pm SD: 66 \pm 40 min per week) compared to their objectively measured accelerometry data, whereas NW women reported less moderate physical activity (mean \pm SD: 29 \pm 52 min per week) compared to their objectively measured accelerometry data.

DISCUSSION

The main finding of the present study was that there were no measurable differences in resting metabolic rate or activity-related energy expenditure between RO women and their NW BMI-matched counterparts. Measures of resting metabolic rate and substrate metabolism, energy and macronutrient intake, eating and exercise behaviors and finally, body shape concern, were largely comparable between groups. As such, these variables display little evidence for any compensatory changes in the RO group in our sample, posing minimal threat to lasting weight loss in formerly overweight women.

Though no physiological differences were observed in our reduced-overweight women (mean initial BMI = 28.3), Liebel et al. (1995) showed that resting energy expenditure decreased significantly in reduced-obese subjects maintaining a weight reduction of $\geq 10\%$ below initial BMI. This may imply that physiological adaptations are more likely to present in those with a higher initial BMI. Interestingly, Chaput et al. (2007) demonstrated that an intervention with an increased dietary protein intake resists these changes and, since our RO women reported a slightly but significantly higher protein intake compared to the NW group, this measure may have afforded some measure of protection against physiological compensation in these women.

Our study revealed that body shape perception between groups was also comparable and that TFEQ scores were low for both groups (scores of 12 \pm 4 and 10 \pm 4 for RO and NW, respectively). These results suggest that, in terms of body concern and eating behaviour, formerly overweight individuals can achieve weight reduction without significant psychological or behavioral compensation. While researchers have previously identified elevated scores for restrained eating in those attempting maintenance after successful weight reduction (Klem et al., 1998), these subjects had lost a mean of 30 \pm 15 kg of body weight, suggesting that eating behaviors become disrupted only with greater degrees of weight reduction. This is comparable to findings of Chaput

Table 4. Self-reported and measured physical activity in RO and NW subjects.

Parameter	RO (n = 25)	NW (n = 19)
Moderate PA (min per wk) (median; 25th;75th quartile)	270(150; 360)	113(20; 255)*
Vigorous PA (min per wk) (median; 25; 75th quartile)	45(0; 180)	60(0; 213)
MVPA (min per wk) (median; 25th;75th quartile)	330(240; 640)	270(90; 408)
Measured Moderate PA (min per week, mean \pm SD) [#]	162 \pm 128	159 \pm 113
Measured Vigorous PA (min per week, mean \pm SD) [#]	33 \pm 34	87 \pm 104
Measured Total MVPA (min per week, mean \pm SD) [#]	189 \pm 137	194 \pm 152
GPAQ-measured MVPA (min per wk, mean \pm SD) [#]	66 \pm 150	-29 \pm 201

*P < 0.05, student t-tests for group differences; MVPA: moderate-to-vigorous PA; # Measured Physical activity determined in subgroups RO (n = 14) and RW (n = 15).

et al. (2007) who suggest that elevated TFEQ scores are more common in individuals who have lost a greater percentage of initial body weight. Finally, rather than being directly causally related to risk of weight gain, increased dissatisfaction with body shape has been described as a precursor of atypical eating behaviors which may alter energy intake and prompt regain (Stice and Shaw, 2002). As described previously, such eating disturbances and heightened concern for body shape were not detected in either of our groups.

Since one of the only distinguishing characteristics between our groups was an over-reporting of moderate intensity exercise by the RO women, we propose the overestimation of physical activity (PA) energy expenditure as the only identifiable factor in the present study that may predispose reduced-overweight women to regain. This does raise some concern as women who over-report physical activity levels have been shown to have an overall lower rate of success at weight loss maintenance than those who accurately predict PA (Jakicic et al., 1998). Finally, although both groups reported "adequate" levels of moderate-to-vigorous physical activity (MVPA) (≥ 150 min/wk) (Haskell et al., 2007), it has been demonstrated that successful weight loss maintainers sustain relatively high levels of weekly PA corresponding to approximately 60 min of moderate intensity exercise per day (Phelan et al., 2006). Using objective measurement, our RO women fell well below this level (27 ± 20 min MVPA/day/wk).

According to Reed et al. (2013), a decline in energy expenditure in the weight-reduced state coupled with a heightened drive to eat is the leading cause of regain following successful weight reduction. Our reduced group did not mirror these adaptations in physiology or behavior, thus we would like to suggest a cluster of factors which may explain the insignificance between our RO and NW women. First, reductions in resting and activity-related energy expenditure have been shown to be less common among those who have: (i) undergone more moderate reductions in body weight from maximum

weight ($\leq 15\%$ versus $\geq 20\%$) (Weiss et al., 2007), (ii) maintained reductions in body weight for longer (≥ 2 versus ≤ 2 years) and (iii) preserved lean mass during and following weight loss efforts (Vogels et al., 2005). Second, increases in hunger and appetite have been demonstrated to be attenuated by an increased protein intake in the reduced weight state (Chaput et al., 2007). Due to the fact that (of our 25 RO women) 17 had lost less than 15% of initial body weight and that a further 9 had maintained their reduced weight for 2 years or longer, one could argue that these factors (in combination with the significantly higher protein intake by this group) may have protected them against the physiological and behavioural compensatory mechanisms encouraging weight regain.

According to Wing and Hill (2001), successful weight loss maintenance is defined as a deliberate $\geq 10\%$ reduction in initial body weight sustained for at least one year. Although the RO subjects reported a mean weight loss of 14% and a mean maintenance time of 2 years, there was large variability in these measures (weight loss ranged from 6 to 25.5%; maintenance time ranged from 2 months to 9 years), which may have decreased the sensitivity of our measures. This is the foremost limitation of our study.

Conclusion

Energy balance (as it relates to resting metabolic rate and activity-related energy expenditure) is comparable in RO and NW women. With the exception of a tendency to over-report moderate physical activity by the RO group, our findings suggest that formerly overweight women were able to maintain weight losses without measurable physiological or behavioral compensation. This finding warrants further investigation as to why weight regain is so common, especially among those who have achieved greater weight losses and those with an initial BMI of 30 or greater.

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Full Length Research Paper

In vivo evaluation of antidiarrhoeal activity of the leaves of *Azima tetracantha* Linn

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The aqueous crude extract of the leaves of *Azima tetracantha* was studied for its phytochemical constituents and antidiarrhoeal activity using castor oil-induced diarrhoea and castor oil-induced enteropooling in rats. The phytochemical studies of the aqueous extract revealed the presence of alkaloids, flavonoids, tannins and saponins. The extract showed significant ($p < 0.001$) protection against castor oil-induced diarrhoea and castor oil-induced enteropooling at (100 mg/kg). The presence of some of the phytochemicals in the root extract may be responsible for the observed effects, and also the basis for its use in traditional medicine as antidiarrhoeal drug.

Key words: *Azima tetracantha*, enteropooling, anti-diarrhoeal.

INTRODUCTION

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain (Ezekwesili et al., 2004). It is a leading cause of malnutrition and death among children in the developing countries of the world today (Victoria et al., 2000). Many governments and international organizations are trying to control this disease but the rate of incidence is still high, about 7.1 million per year (Park, 2000). Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhoea but they have some side effects. The natural drugs are used as antidiarrhoeal drugs, which are not always free from adverse effects (Hardman and Limberd, 1992). Therefore, the search for safe and more effective agents has continued to be an important area of active research. Since ancient times, diarrhoea has been treated orally with several medicinal plants or their extracts based on folklore medicine.

Azima tetracantha (Salvadoraceae) is known as 'Mulsangu' in Tamil and 'Kundali' in Sanskrit, respectively. Its root, root bark and leaves are used with food as a remedy for rheumatism (Kirtikar and Basu, 1984). It is a

powerful diuretic given in rheumatism, dropsy, dyspepsia and chronic diarrhoea and as a stimulant tonic after confinement (Nadkarni, 1976). *A. tetracantha* as efficient acute phase anti-inflammatory drug is traditionally used by Indian medical practitioners (Ismail et al., 1997). *A. tetracantha* is used to treat cough, phthisis, asthma, smallpox and diarrhoea. The decoction of the stem bark is considered astringent, expectorant and used for fevers (Reddy et al., 1991). The present study was undertaken to evaluate the antidiarrhoeal potential of aqueous extract of leaves of *A. tetracantha* in normal and castor oil induced diarrhoeal rats.

MATERIALS AND METHODS

Plant

The fresh leaves of *A. tetracantha* (Salvadoraceae) were collected from the thanjavur. The collected leaves were identified and authenticated by a Botanist, Prof. Dr. M. Jegadesan, Department of Herbal and Environmental science, Tamil University, Thanjavur, Tamil Nadu. A Voucher B specimen (Specimen no: 26) was been

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Table 1. Preliminary phytochemical analysis of *Azima tetracantha* leaves crude extract.

Phytoconstituent	Tannin	Alkaloid	Flavonoid	Saponin	Glycoside
Aqueous extract	+	+	+	+	-

deposited at the Herbarium of the department. Leaves were dried in shade for 20 days and then powdered to get a coarse powder. This powder was stored in air tight container and used for further successive extraction.

Preparation of crude extract

The leaves were air-dried and ground into powder. A total of 200 g of the powdered leaves were macerated in 500 ml of distilled water for 8 h and filtered through cheese cloth, glasswool and Whatman No. 1 filter paper. The filtrate was evaporated to dryness by heating in a water bath at a temperature of 100°C. The dry aqueous extract was stored in the refrigerator and used as the crude extract.

Animal

Healthy male albino rats (*Rattus norvegicus*) of weight 150 to 200 g, were obtained from the Animal House of the Tamil University. All the animals were housed in a clean and well-ventilated house conditions [temperature $23 \pm 1^\circ\text{C}$; photoperiod (the interval in a 24-h period during which an organism is exposed to light): 12 h natural light and 12 h dark; humidity: 45 to 50%]. They were also allowed free access to Balanced Trusty Chunks (Sai Durga Foods Ltd, Bangalore) and tap water. The cleaning of the cages was done daily.

Castor oil induced diarrhea

Animals were divided into three groups (n = 6). The first group of animals, which served as control was administered with castor oil (1 ml/rats). The second group castor oil (1 ml/rats) + crude powder (100 mg/100 g body weight). Third group received standard drug, loperamide (3 mg/kg) orally as suspension.

Phytochemical screening

The aqueous crude leaves extract of the plant was subjected to qualitative chemical screening for the identification of the tannins, alkaloids, flavonoids, saponin and glycosides using standard procedures (Trease and Evans, 1996).

Castor oil-induced diarrhoea in rats

A total of 18 albino rats (*R. norvegicus*) were divided into three groups of six animals in each. All rats were fasted for 18 h and received castor oil at a dose of 1 ml/animal orally (p.o.) using orogastric cannula for induction of diarrhea (Doherty, 1981). Thirty minutes after castor oil administration, rats of group I (control) received 1.0 ml/100 g of 0.9% NaCl in distilled water (normal saline) and rats of groups II, received 100 mg/kg of *A. tetracantha* leaves crude powder extract p.o. and group III received standard drug, loperamide (3 mg/kg p.o.), respectively. The animals were placed separately in metabolic cages over white clean Whatman filter paper which was changed every hour. The severity of diarrhoea was assessed each hour for 4 h. The total number of diarrhoea feces of the control group was considered 100%.

$$\% \text{ Inhibition} = (\text{Control} - \text{Test}) \times 100/\text{Control}$$

Castor oil-induced enteropooling

Castor oil-induced enteropooling was determined by the method of Robert et al. (1976). The adult rats (*R. norvegicus*) selected without sex discrimination were fasted for 18 h and divided into three groups of six animals each. Castor oil (1 ml) was administered orally to these animals. One hour later, Group I received 1 ml/100 g of normal saline solution and rats of groups II received 100 mg/kg *A. tetracantha* leaves crude powder p.o and group III received standard drug, loperamide (3 mg/kg orally), respectively. After 2 h of treatment, the rats were sacrificed by ether anesthesia. The edges of the intestine from pylorus to caecum were tied with thread and the intestine was removed and weighed. Intestinal content was collected by measuring cylinder, and volume measured.

Statistical analysis

Results are presented as means \pm standard deviation (SD) and simple percentages. The student 't' test was used to determine the significant difference between two groups ($p < 0.001$).

RESULTS

Phytochemical analysis

The phytochemical results confirm the presence of alkaloids, flavonoids, tannins and saponins in extracts but in variable quantities (Table 1). These are the phytochemicals which are essential in many medicinal plants responsible for the antidiarrhoeal (Patricia et al., 2005). The reported medicinal property of the plant might be due to the presence of these bioactive components in *A. tetracantha*. Phytochemical such as glycosides, were found to be absent in the extract.

In the castor oil-induced diarrhoea experiment, aqueous extract of *A. tetracantha* produced a markedly antidiarrhoeal effect in the rats, as shown in Table 2 (Figure 1). At dose of 100 mg/kg, the extract significantly decreased ($p < 0.01$) the total number of watery stool produced upon administration of castor oil (4.84 ± 0.31 at 100 mg/kg) compare to the control group (22.00 ± 0.90). The effect of the dose of the extract was similar to that of the standard drug, loperamide (3 mg/kg). *A. tetracantha* leaves crude extract significantly ($p < 0.001$) inhibited castor oil-induced enteropooling in rats at oral dose 100 mg/kg (Table 3 and Figure 2). The intestinal fluid in control animals was 3.29 ± 0.06 ml. The inhibition of intestinal accumulation was 78% ($p < 0.001$) at dose 100 mg/kg of the drug. The standard drug, loperamide (3 mg/kg) also significantly inhibited ($p < 0.001$) intestinal

Table 2. Effect of *Azima tetracantha* leaves crude extract on castor oil induced diarrhea.

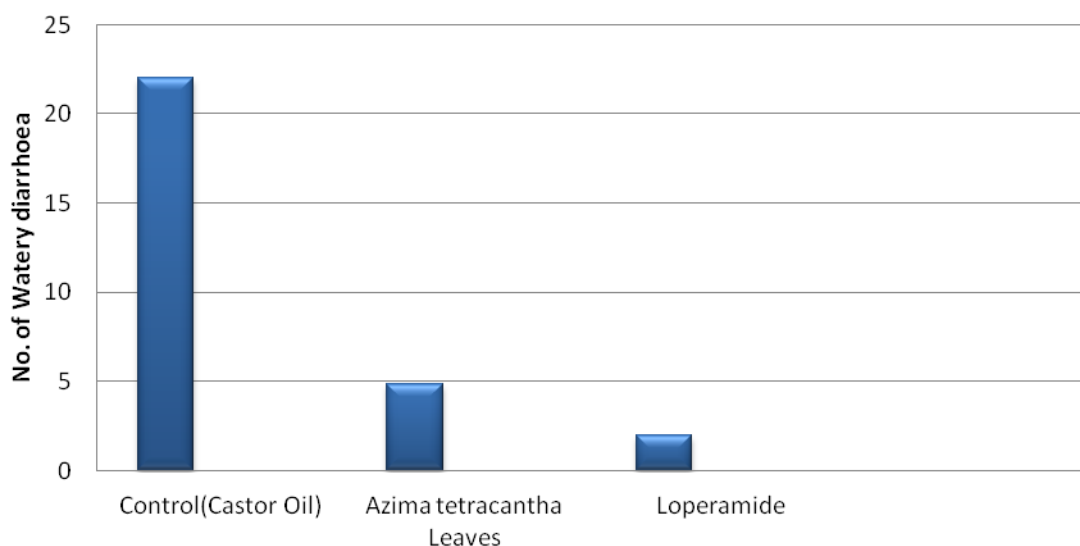
Subject	Dose (mg/kg)	No. of watery stool diarrhea	% Inhibition
Control (castor oil)	1 ml	22.00±0.90	--
<i>Azima tetracantha</i> leaves crude extract	100	4.84±0.31***	78
Loperamide	3	2.00±0.26***	90

Values are expressed as mean ± SD. n = 6, *** P< 0.001 compared with control.

Table 3. Effect of extract of *Azima tetracantha* Leaves crude extract on castor oil-induced enteropooling.

Subject	Dose (mg/kg)	Fluid volume (ml)	% Inhibition
Control (castor oil)	1 ml	3.29±0.06	--
<i>Azima tetracantha</i> leaves crude extract	100	0.74±0.02***	78
Loperamide	3	0.43±0.01***	87

Values are expressed as mean ± S.D. n = 6, *** P< 0.001 compared with Control

**Figure 1.** A graph of the effect of *Azima tetracantha* leaves crude extract on castor oil induced diarrhea.

fluid accumulation (87%).

DISCUSSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by rush resulting in an excess loss of fluid in the faeces. In some diarrhea, the secretory component predominates while other diarrhoea is characterized by hypermotility (Chitme et al., 2004). Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid (Ammon et al., 1974; Watson and Gordon, 1962) which stimulates peristaltic activity in the small

intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin (Galvez et al., 1993). Castor oil reported to induce diarrhoea by increasing the volume of intestinal contents by preventing the re-absorption of water. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin (Pierce et al., 1971).

The results of this study revealed that the aqueous leaves extract of *A. tetracantha* produced statistically significant protection against diarrhoea and was found to be comparable to loperamide; a drug widely employed against diarrhea disorders which effectively antagonizes

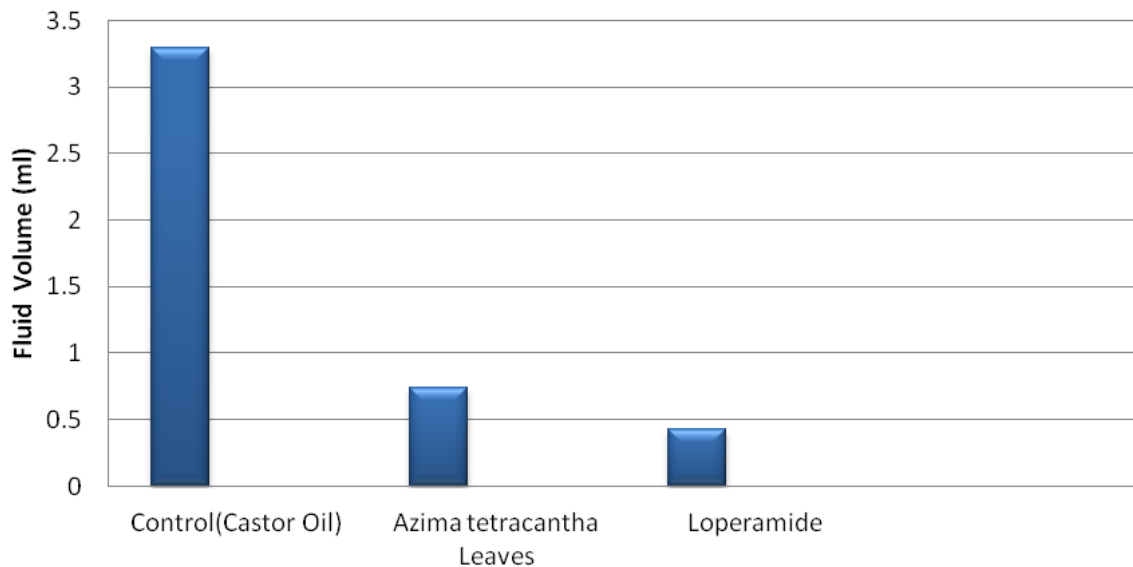


Figure 2. A graph of effect of *Azima tetracantha* Leaves crude extract on castor oil-induced enteropooling.

diarrhoea induced by castor oil, prostaglandin and cholera toxin (Niemegeers et al., 1974; Karim and Adeikam, 1977; Facack et al., 1981). The pharmacological effect of loperamide is due to its antimotility and antisecretory properties (Couper, 1987).

The anti-diarrhoeal activities of medicinal plants have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids and terpenoids (Havagiray et al., 2004). While the flavonoids are known to inhibit intestinal motility and hydroelectrolytic secretion (Venkatesan et al., 2005), tannins denature proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion (Havagiray et al., 2004). Therefore, the anti-diarrhoeal activity of *A. tetracantha* leaves crude extract observed in this study may be attributed to the presence of tannins, flavonoids, alkaloids and saponins in the crude extract.

Conclusion

The anti-diarrhoeal activity of *A. tetracantha* leaves crude extract observed in this study may be attributed to the presence of tannins, flavonoids, alkaloids and saponins in the crude extract. The prolonged onset of diarrhoea, inhibition of castor oil-induced enteropooling and the suppressed propulsive movement observed in this study are indications of anti-diarrhoeal potential of *A. tetracantha* leaf crude extract. Further studies are however needed to establish the safety of the extract and to possibly isolate the active principle responsible for the observed effects.

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UPCOMING CONFERENCES

XVII International Congress on Nutrition and Metabolism in Renal Disease, Würzburg, Germany

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