Using The BALB/c Asthmatic Mouse Model to Investigate the Effects of Hydrocortisone and a Herbal Asthma Medicine on Animal Weight

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Summary
Euphorbia hirta is an anti-inflammatory phytomedicine used to treat asthma. It contains, amongst other components, Quercitrin, which is converted to Quercetin in the alimentary canal and is thought to be responsible for its therapeutic potential. In this study, the BALB/c asthmatic mouse mode was used to investigate the short and long term effects of *Euphorbia hirta* on asthma and weight change. Hydrocortisone was used as positive control. Data showed that animals in both the short and long term control groups experienced slow but steady progressive weight increments. Both immunization and nebulization had positive weight gain effects on the animals but the effects were more pronounced following immunization but were only minimal following nebulization. Prolonged treatment with hydrocortisone remarkably reduced the cumulative weight gained following prior experimental procedures (immunization and nebulization), followed by a slow and sustained increase in the rate of weight gain. On the other hand, prolonged administration of the plant causes only a minimal reduction in weight gained due to induced asthmatic conditions. It is concluded that *E. hirta*, besides reducing asthma symptoms similarly to that of hydrocortisone (as seen with white blood cell counts), does not impact on weight gain as severely as hydrocortisone.

Introduction
The relationship between airway inflammation (including that seen in asthma) with either excessive body weight, anomalous body mass index, or obesity has been reported (*Camargo et al, 1999; Hakala et. al, 2000*; *Aaron et al. 2004; Weiss & Shore, 2004; Beuther et al, 2006*) but the underlying mechanisms remain obscure. Although it is known that obesity worsens asthmatic conditions, the cause-effect relationship (i.e. whether one of these two conditions can lead to the other) is not understood. Shore (*2006*), in a review article, argued that the relationship between asthma and weight changes as reported in most previous studies, does not address the direction of causality, adding that one possible interpretation of these studies could be that asthma leads to obesity, perhaps because asthmatics adopt a sedentary lifestyle to avoid respiratory symptoms during exercise. A different opinion by Hayman, (*2006*) was that any factor that causes inflammation could lead to weight gain that could in turn lead to more inflammation. In adult humans, a reduction in excessive body weight by medical treatment and surgical procedures has resulted in a reduction of asthma symptoms, medication usage, and severity, and in improvement of lung function, indicating a possible causal relationship (*Macgregor & Greenburg, 1993; Dixon et al, 1999; Stenius-Aarniala et al, 2000*).

Animal models of mice, guinea pigs, rats, dogs, cats, monkeys, sheep, and horses have been developed to

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study disease pathogenesis and for drug discovery (*Epstein, 2004a, b*). Since the first demonstration of allergic mouse asthma was reported in 1994, mice have become one of the most extensively studied model systems (*Epstein, 2006*). BALB/c mice were used in this study primarily because so much is already known about their immune responses and genetics from literature. Results from previous studies have shown that despite a few shortcomings, the BALB/c mouse model still manages to paint a good pattern of the human airway disease better than any other model (*Gleich et al, 1988; Zhao et al, 2000; Blyth et al, 2000; Leigh et al, 2002; Mcmillan & Lloyd, 2004; Johnson et al, 2004; Jungsuwadee et al, 2004*).

Animal models of weight studies have also been reported in literature (*Harris et al, 1998; Snibson et al, 2005; Retana-Ma´rquez et al, 2003*) and the use of different plant extracts or herbal preparations in managing weight changes have also been reported. For instance, a study using plant extracts showed that the leaves of *Syzygium cordatum* did not cause any weight changes in diabetic rats (*Musabayane et al, 2005*) unlike the significant weight gains reported in ovariectomised mice treated with extracts from the plant *Onobrychis ebenoides* (*Dontas et al, 2006*). Another study (*Jung et al, 2004*) showed that different medicinal plant extracts did not have significant effect on the body weight compared to the control group.

In this study, the possible effects of the plant extract *Euphorbia hirta* on asthma and weight change was investigated. *Euphorbia hirta* (Euphorbiaceae) is found worldwide and in many parts of Africa. Extracts or the decoction of the flowering and fruiting plant have long been used (and are still being used) in East and West Africa for the treatment of asthma and respiratory tract infections and are sometimes combined with bronchial sedatives like *Grindelia robusta* in preparations for inhalation (*Oliver, 1959; Kokwaro, 1976*). *E. hirta* is also used for the treatment of coughs, chronic bronchitis and pulmonary disorders; for relieving hay fever and catarrh; as an anti-hypertensive agent, analgesic, antipyretic and sedative; and the diuretic properties of the plant have also been reported (*Dickshit, 1943; Hazleton & Hellerman, 1954; Watt & Breyer- Brandwijk, 1962; Le Strange, 1977; Wong, 1980; Lanhers, 1990; 1991*).

The exact mechanisms by which *E. hirta* relieves asthma are not clear, but significant and dose-dependent anti-inflammatory effects have been observed (*Martinez-Vazquez et al, 1999*). The medicinal properties of *E. hirta* are, however, possibly due to its content of many active ingredients including alkaloids, flavonoids, glycosides, sterols, tannins and triterpenoids (*Gupta & Garg, 1966; Atallah & Nicholas, 1972; Sofowora, 1984; Galvez et al, 1992*). Flavanoids are well known to have a high antioxidant activity (*Kandaswami & Middleton, 1994*). The (bio)flavonoid in *E. hirta*, Quercitrin (3-rhamnosylquercetin) is usually converted to Quercetin (3-O-alpha-L-rhamnopyranoside - Quercetrin) in the alimentary canal and appears to be the compound that has given this plant its great therapeutic potential. Quercitrin is the glycosylated form of Quercetin and possesses antioxidant as well as anti-inflammatory properties (*Comalada et al, 2005*). Another flavonoid in *E. hirta,* Myricitrin also seems to be a powerful Nitric Oxide Synthase-inhibiting antioxidant. In addition, the sterols 24-methylene-cycloartenol and β-sitosterol have been reported to exert significant and dose-dependent anti-inflammatory activity while the triterpene β-amyrin also showed anti-inflammatory effects (*Martinez-Vazquez et al, 1999*).

Free radicals are created in the body as a by-product of energy released by cells. Excessive amounts of free radicals can cause a wide range of diseases but antioxidants are known to help the body fight and neutralise these reactive groups. Asthma has long been associated with an overall increase in reactive groups and oxidative stress (*Barnes, 1990; Kharitinov et al, 1994; Nadeem et al, 2003*). It could be concluded that one way by which *E. hirta* functions for the treatment of asthma is probably through synergistic anti-inflammatory

and antioxidant activities of especially the flavonoids, sterols and triterpenoids (*Park & Lee, 2006)*. Since many weight dynamics, in especially asthmatics, are related to oxidative stress (*Fenster et al, 2004; Johnson et al, 2007*) the presence of antioxidants in *E. hirta* could have positive effects in eliminating free radicals associated with weight processes in animals treated with extracts of this plant.

A number of previous studies on the direct or indirect effects of drugs on weight changes have been reported. Hausberger & Hausberger, *(1958)* reported that 60% of male Wistar rats which received cortisone (5 mg/day) had diminished gain of body weight and total body protein just as a marked decrease in weight was observed in another study in mice receiving hydrocortisone (*Borovitskaya et al, 1971*). Also, following administration of a high-dose of systemic dexamethasone for 3 days, Kumar et al, (*1997*) reported a marked catabolic effect with weight loss in rats. In another study, prednisolone was found to reduce body weight in mice and guinea pigs (*Nagao et al, 2004*) while other studies with antipsychotic drugs have shown varying results (*Ganguli, 1999; Goudie et al, 2002*).

In view of the above, the current study involved the use of the BALB/c mouse model to investigate the possible effects of hydrocortisone and *E. hirta* on weight changes in asthmatic mice. Animals were proved asthmatic following analysis of blood cell count as reported in our previous article (*Pretorius et al, 2007*). *E. hirta* also proved to effectively reduce white blood counts to the same level as that of hydrocortisone (*Pretorius et al, 2007*), suggesting that it is an effective treatment for asthma.

Materials and Methods

Balb/c mice

A total of sixty (60) six-week-old male BALB/c mice of mean weight 20g were used in this study. All the animals were obtained from the Biomedical Research Centre in the Faculty of Veterinary Sciences of the University of Pretoria and maintained in a pathogen-free environment at the Onderstepoort Animal Care facility in Pretoria. Polycarbonate Type III cages were obtained from Tecniplast. A temperature range of 20-24°C, a relative humidity of 40-60% and a 12-hour daylight and 12-hour night were maintained. Only one mouse was housed per cage, and autoclaved wood shavings were used as bedding while white facial tissue paper was added per cage for enrichment especially to reduce male mouse aggressive behaviour during handling.

Animals were provided with OVA-free food (Balanced EpolT mice cubes and pellets, (obtained from EPOL - a division of Rainbow Farms PTY LTD, South Africa) and pre-boiled tap water *ad libitum,* one bottle per cage. Mice were allowed to acclimatize for seven days before the experiments commenced. All experimental protocols employed complied with the requirements of the University of Pretoria's Animal Use and Care Committee (UPAUCC).

Hydrocortisone

Hydrocortisone was used in this study as a pharmaceutical control for the extracts of *Euphorbia hirta*. Two milligrams (2mg) of the sterile powder (Brand name Solu-Cortef®), which contains hydrocortisone sodium succinate as the active ingredient, was dissolved in 100ml of bacteriostatic injection water, giving a concentration of 50mg/ml. The drug was purchased from Elwierda Apteek/ Pharmacy Wirdapark, Pretoria and manufactured by Pharmacia South Africa (Pty) Ltd, South Africa. In this study, a high dose (125mg/kg) and a low dose (100mg/kg) of the 50mg/ml hydrocortisone preparation were used. Fresh solution was prepared for each injection.

Euphorbia hirta

Euphorbia hirta obtained from fields in the Gezina area of Pretoria, South Africa was identified and verified by staff in the Department of Botany, University of Pretoria, South Africa by comparison with an authentic voucher specimen of the plant

available at the H.G.W.J. Schweikerdt Herbarium of the University.

The plant extract of *E hirta* was prepared for the entire plant including the leaves, stem and roots of the mature plant. The plant material was air-dried, ground into a fine powder and 50g of this material was extracted in 500ml boiling double-distilled water (100mg/ml) for 20 minutes with continues stirring. The water extract was allowed to cool, filtered and then dried using a rotary evaporator at 40°C. The final dry mass was 3000mg (6% of plant material extracted). A stock solution of 60 mg/ml (8.33 times concentrated) was prepared and stored at -70°C. Mice with an average mass of 20g were divided into two groups and received either (i) 62.5mg/ml (1.25mg/mouse, equivalent to 10mg or 1.66ml of the water extract derived from 166mg dried plant material) (ii) 25 mg/kg (0.312mg/mouse, equivalent to 4mg or 0.66ml of the water extract derived from 66mg dried plant material).

Only the aqueous extract of the plant was used for the animal studies as commonly prepared by traditional healers. The aboveground parts of the plant were allowed to dry at room temperature for one week and then the plant material was ground into a fine powder. Fifty grams (50g) of the sample were extracted in 500ml of double distilled water after which it was filtered and dried on a rotary evaporator at 40 °C. A stock aqueous solution of 50mg/ml of the resulting plant extract was prepared and stored in a fridge until used. A high dose (62.5mg/kg) and a low dose (25mg/kg) of the plant extract were administered orally to the mice depending on the group as follows.

Animal care and grouping

The mice were divided into two main groups, one for the short-term (ST) and one for the (LT) long-term phases of the study. Each main group consisted of thirty (30 animals) and further divided into (6) sub-groups according to the treatment to be given – control (CT), asthma (AS), high hydrocortisone-treated (HC), low hydrocortisone-treated (low HC), high *E. hirta* - treated (high *EH*) and low *E. hirta* - treated (low *EH*) groups respectively.

The process of inducing asthma involved sensitisation (immunization) and nebulization (allergen challenge). The study was extended beyond day 18 (when the short-term phase ended) to evaluate the effects of long-term exposure of the mice to the test agents (hydrocortisone and *Euphorbia hirta* extracts). All animals except those in the CT group were sensitized and nebulized before treatment with the two test agents. Asthma was induced in the AS group and the animals were left untreated whereas animals in all other test groups were treated with either the plant extract or hydrocortisone after they were exposed to the same asthma-inducing conditions as the animals in the AS group.

Sensitization

Sensitization was done on days 0 and 5 respectively via intraperitoneal injection of a solution of 25mg OVA (ovalbumin; grade V; Sigma-Aldrich) emulsified in 2mg aluminium hydroxide [Al $(OH)_3$] and dissolved in 0.5ml of 0.9% saline solution. All mice except those in the CT groups were sensitized and allowed duration of one week before exposure (challenge).

Nebulization

Nebulization was carried out one week after immunization and involved placing the mice (except those in the CT groups) in a Plexiglas chamber and exposing them to fumes generated via a KLAVA ultrasonic nebulizer from a 1% OVA in PBS (phosphate buffered saline) solution (1mg OVA in 100 ml PBS). In order to induce acute onset of asthma, mice were nebulized for two consecutive 30-minute periods daily, with a one-hour interval, on days 13, 14 and 15 for the short-term study and repeated on days 34, 35 and 36 for the long-term study. Animals were proved asthmatic following analysis of blood cell count as reported in our previous article (*Pretorius et al, 2007*).

Administration of test agents

A hydrocortisone **(**HC**)** dose range of 75mg/kg (low) and 125mg/kg (high) was used in this study and administered to eligible short-term and long-term animal study groups via intraperitoneal injection. On the other hand, the high (62.5mg/kg) and low (25mg/kg) doses of the plant extract *(EH)* were administered orally to the mice (short and long-term groups). Each animal received the same dose of the treatment agents twice daily, with an hour's interval between treatments.

For groups involved in the short-term studies, administration of treatment agents was done on days 15 (about 30 minutes after last nebulization), 16 and 17. Animals involved in the short-term studies, were terminated on day 18 to end the short-term studies. For groups involved in the long-term studies, administration of treatment agents was on days 15 (about 30 minutes after the last batch of nebulization), 16, 17, 18, 22, 25, 29 and 32. Treatment was stopped after day 32 to repeat nebulization (on days 34, 35, 36) and was continued daily for one week from days 39, 40, 41, 42, 43, 44 and 45.

All treated animals were also observed for basic asthmatic symptoms (wheezing and difficulty to breath) shortly after nebulization, 10 minutes before treatment as well as one hour after each treatment exposure. Skilled UPBRC technical personnel terminated the long-term animals on the morning of Day 46 via bleeding them to death and cervical dislocation.

Procedures for animal weight result analysis

All observations for basic asthmatic symptoms including reduced physical activity, general discomfort, difficulty of breathing and wheezing, were made throughout the study by the principal investigator to avoid bias. Although these symptoms were present in most of the animals just after the nebulization procedure, they did not cause much damage and eased off gradually. Greater improvement was however observed after about 30 minutes following treatment with either hydrocortisone or the plant extract.

Daily weighing was done until day 46 when the last batch of animals was terminated. The mean weights (in grams) for all the groups on each day as well as the corresponding values for standard deviation were determined and expressed in grams ± standard deviation.

Mean weight values on day 1 were considered baseline weights and a 2-tailed paired-sample t-test was used to determine differences between the baseline weights and other weight values recorded on all the reference days in each group. Since the starting weights varied between the groups, mean weight values per group per day were converted into percentages of the starting weights and the respective percentage weight differences (PWDs) were determined relative to the starting or baseline weights. This offered a more appropriate means of determining weight differences between groups as opposed to using the original weight values. Comparison was made between the different experimental groups on the chosen reference days of experimental intervention to determine differences in weight change patterns.

Choice of reference days on which to analyze and study the possible effects of the different experimental interventions was informed by a number of factors. The selection took into account the days on which animals were sensitized (days 0 and 5), nebulized (days 13-15) as well as treated (days 15-17 for ST studies; days 22, 25, 29, 32, 39, 40, 41, 42, 43, 44, 45 for the LT studies respectively). The reference days chosen include days 6, 13, 15, 18, 26, 32, 37, 42 and 46 (Table 1).

Day 6 is one day after the last immunization and therefore the acute effects of immunization could be determined on this day. Day 13 was analyzed because any possible effects of the immunization procedure on changes in animal weights could appear on this day. Similarly, day 15 was considered because it is the last day of the first batch of nebulization and the day when administration of hydrocortisone and the plant extract commenced. On this day, the acute effects of nebulization on weight changes could be determined. Finally, the weights recorded on day

Table 1: Day of weighing and expected effects on weight changes

DAY	Expected effects of procedures on weight changes
1	Baseline weight
6	Early effects of immunization
13	Late effects of immunization (just before first nebulization)
15	Effects of first nebulization
18	Effects of first batch of treatment on nebulization (just before sacrifice)
26	Midstream effects of continuous treatment
32	Late effects of continuous treatment
37	Acute effects of repeated nebulization on weight changes
42	Early effects of post-nebulization treatment on weight changes
46	Terminal effects of post-nebulization treatment on weight changes

18 (the last day of the ST studies) could provide information about the effects of all treatments given on days 15, 16 and 17.

Days of analysis for the LT studies included days 18, 26, 32, 37, 42 and 46 since administration of treatment agents continued on days 22, 25, 29, 32 and later days 39, 40, 41, 42, 43, 44, 45. Day 18 was studied to assess the early effects of treatments on animal weights. Day 26 was chosen to assess the midterm effects while day 32 was chosen to assess the late effects of treatments. Since treatment was suspended while the second batch of nebulization was done on days 34-36, treatment effects evaluated after day 36 will be related to the reciprocal effects of nebulization and treatment on the animal weights.

Results

Tabulated summaries for mean weights of animals in the ST and LT study groups are given in Table 2 below. Intra-group comparisons show that most of the mean weights in each experimental group are significantly lower ($p<0.05$) than the mean baseline or starting weights of same group (marked with asterisks).

Comparisons between respective groups are based on evaluation of how closely the values in all groups compare to the values in the reference group (mostly the controls) as seen in Tables 3 as well as from the graphs (Figures 1 and 2). The absolute mean weight values were not used when comparing data by group since the starting weights vary between groups. Instead, respective percentage values were determined in relation to the baseline weights and were then compared. Values for the percentage weight differences from baseline weights (PWDs) were obtained using the formula:

$$PWD = \frac{[\text{Mean weight}_n - \text{Mean weight}_b]}{\text{Mean baseline weight}_b} \times 100$$

Where mean weight_n = mean weight of specified group on selected day and mean weight_b = mean baseline weight of the specified group. Thus, all baseline weights became equivalent to 100% and all original mean weight values greater than the baseline weights became percentage values higher than 100% and vice versa for all lower original mean weight values. The respective change in percentage weights between groups on the same reference day were then compared by evaluating their closeness to (or their comparability with) the percentage weight values of the group under reference (Tables 3 below).

Discussion

Weight loss or gain can be caused by a number of factors including motivation, eating behaviour, amount of activity (especially exercise), overall health, metabolism, stress, etc. Excessive body weight increases the risk of asthma (Camargo et

Table 2: Intra-group mean weights on selected days for ST and LT study

ST DAY	Mean Weights(g) ± SD					
	Control	Asthmatic mice	High dose cortisone	Low dose cortisone	High dose *E. hirta*	Low dose *E. hirta*
1	22.47±0.71	18.29±1.85	21.43±1.53	18.47±2.82	18.89±0.76	17.95±2.56*
6	22.59±0.75	19.57±1.50*	22.23±1.26*	20.69±0.98*	19.59±0.66*	19.42±2.36*
13	23.01±0.64	20.62±1.42*	22.87±0.93*	21.19±0.72*	20.23±0.76*	20.26±2.25*
15	23.40±0.61*	20.98±1.33*	23.43±0.97*	21.72±0.64*	20.83±0.49*	20.84±2.24*
18	23.63±0.57*	21.10±1.81*	22.18±0.97*	19.85±0.82	20.22±0.27*	20.51±2.41*

LT DAY	Mean Weights(g) ± SD					
	Control	Asthmatic mice	High dose cortisone	Low dose cortisone	High dose *E. hirta*	Low dose *E. hirta*
1	19.86±2.36	19.08±2.36	18.40±2.40	18.78±1.71	18.07±1.44	20.09±2.34
6	19.92±2.30	20.17±2.09*	19.47±2.00*	19.69±1.20*	19.19±1.54*	20.82±2.05*
13	20.53±2.46*	21.16±1.80*	20.39±1.77*	20.19±1.27*	19.88±1.55*	21.87±2.07*
15	20.74±2.19*	21.61±1.73*	21.07±1.93*	20.51±1.08*	20.27±1.55*	22.14±2.08*
18	21.11±2.12*	21.90±1.73*	19.79±1.44*	19.64±1.03	20.34±1.48*	22.15±2.13*
26	21.83±2.00*	22.63±1.42*	19.83±1.71*	19.94±1.09*	20.80±1.42*	21.96±1.78*
32	22.56±1.92*	23.52±1.43*	20.73±1.58*	20.72±1.15*	21.78±1.46*	23.50±2.16*
37	22.69±2.08*	23.53±1.43*	21.13±1.57*	21.00±0.87*	22.10±1.56*	23.79±2.15*
42	23.04±2.18*	23.87±1.43*	21.26±1.76*	21.54±1.28*	22.61±1.49*	24.21±2.40*
46	22.86±1.98*	24.46±1.64*	20.90±1.22*	21.31±1.32*	23.09±1.32*	24.37±2.31*

*difference significant compared with weight on day 1 (baseline) in same group

al, 1999) and obese individuals with asthma may improve their lung-function symptoms and overall health status by engaging in a weight loss program. A controlled study found that weight loss resulted in significant decreases in episodes of shortness of breath, increases in overall breathing capacity, and decreases in the need for medication to control symptoms (Stenius-Aarniala et al, 2000).

The effects of various forms of activity (experimental procedures, food intake, exercise, physical and chemical stress, etc.) on weight changes have been studied in humans and animals alike. Findings from these studies tend to suggest that weight changes that occur especially in relation to stress are usually in response to internal changes in animal physiology as induced by the stress exposure (*Retana-Ma´rquez et al, 2003*). In one study using restraint stress, rats lost weight and remained hypophagic until a few days after the stressor had ended (*Harris et al, 1998*). In yet another study, stress effects on body weight were observed only with repeated exposure to the stressors, and less body weight gain (but not body weight loss) was observed compared to the control group in animals subjected to stress by immobilization or by immobilization plus tail shocks during three days. The loss in body weight observed in other studies by Ottenweller et al *(1992)* and Marti et al *(1999)* was due to a decrease in food intake.

Table 3: percentage weight differences from baseline weights (PWD)

ST Days	Control	Asthmatic mice	High dose cortisone	Low dose cortisone	High dose *E. hirta*	Low dose *E. hirta*
1	100.00	100.00	100.00	100.00	100.00	100.00
6	100.54	106.99	103.70	112.03	103.72	108.18
13	102.39	112.72	106.70	114.73	107.11	112.82
15	104.15	114.73	109.31	117.60	110.29	116.07
18	105.15	115.39	103.47	107.45	107.08	114.28
LT Days	Control	Asthmatic mice	High dose cortisone	Low dose cortisone	High dose *E. hirta*	Low dose *E. hirta*
1	100.00	100.00	100.00	100.00	100.00	100.00
6	100.30	105.69	105.82	104.86	106.22	103.60
13	103.34	110.86	110.79	107.50	110.04	108.87
15	104.42	113.25	114.52	109.23	112.21	110.19
18	106.28	114.74	107.55	104.57	112.56	110.22
26	109.92	118.60	107.76	106.16	115.13	109.31
32	113.56	123.23	112.64	110.33	120.54	116.97
37	114.21	123.31	114.83	111.84	122.32	118.43
42	115.97	125.06	115.55	114.72	125.13	120.51
46	115.08	128.18	113.60	113.46	127.81	121.30

During most of this study, animals experienced consistent weight increase but there were periods of weight reduction after weight gain. Some of the observed weight gain in all the groups in this study could be attributed to a number of factors including the various experimental interventions. Specifically, any weight changes in the control groups at any time during the study were assumed to be due to changes in normal body metabolic responses, growth as well as changes in food and fluid consumption patterns. This is because control animals had no form of experimental intervention and were provided food and water *ad libitum*.

One interesting observation was that weights in the control and asthma groups were generally among the lowest throughout the study, suggesting that the progressive higher animal weights observed in all other groups were caused by the different experimental procedures. Also, during most of the long-term study period the animals in the asthma group had the highest weights of all the groups.

The respective comparisons made are discussed in the sections below.

Analysis of the effects of different experimental procedures on weight changes during selected days

Early and late effects of immunization

Any possible effects of immunization on weight change could be evaluated on day 6. Since all animals received treatment only on a later day, any exaggerated experimental effects on day 6 would only be due to the immunization procedure. Analyses of animal weights in all the groups show that the mean weights on day 6 were significantly higher than baseline weights (Table 2). Unlike all other weight values on the same day, values for the controls in both the short-term and long-term study groups did not differ significantly from the respective

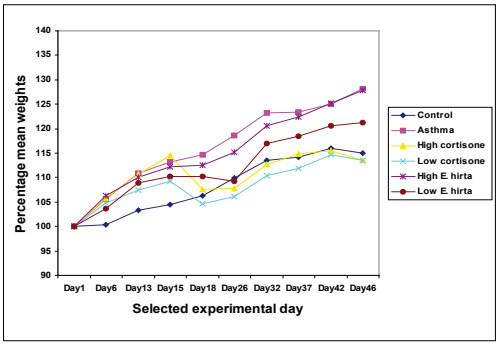

Figure 1: Column graph illustrating weight changes during the ST study

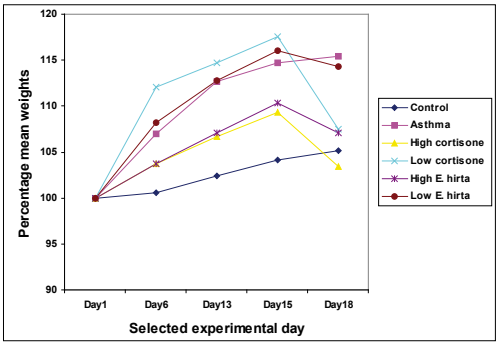

Figure 2: Column graph illustrating weight changes during the ST study

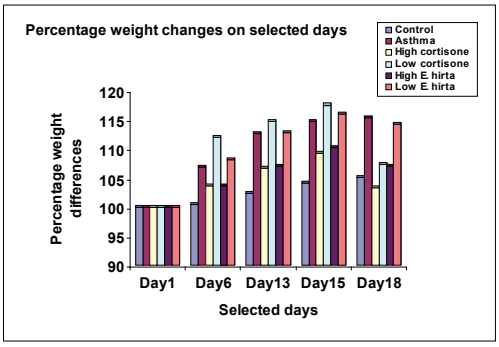

Figure 3: Column graph illustrating weight changes during the LT study

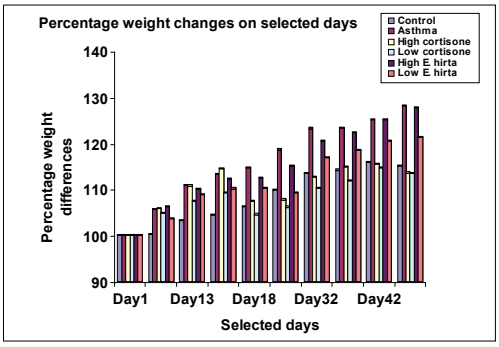

Figure 4: Line graph showing percentage weight changes in the LT group

baseline weights. Thus, the segment in the line graphs for the controls corresponding to the period after immunization is relatively horizontal (Figures 3 and 4). Since line graphs for all other groups show remarkable percentage weight increase, it could be deduced that the early sensitization (immunization) procedure caused some weight gain in the mice.

Similarly, it is reasonable to examine the possible late effects of immunization on animal weights on day 13 because one week's interval was allowed between the first immunization procedure on day 5 and the next nebulization on day 13. Results show that on this day, there was a marked weight gain in most of the groups but a weight gain was also observed in the (unexposed) long-term controls. We suggest that the general weight gain observed may be due to the immunization procedure.

Previous studies involving different forms of animal sensitization showed mixed results, most of which seemed to be species and strain-specific (*Saldanha et al, 2004; Curtis et al, 1990; Huneau et al, 1991*).

Effects of first nebulization

Nebulization was a very important experimental procedure in this study intended to induce the onset of asthma. The first batch of this procedure was on days 13, 14 and 15 before treatment on day 15. The acute effects of nebulization on animal weights could therefore be determined from the weight data taken on day 15 before treatment commenced.

Results from Table 2 show that weights in all the groups on this day were significantly ($p<0.05$) higher than baseline weights. Figures 3 and 4 show that the line graphs for all the groups in both the short and long-term studies were almost horizontal between day 13 and 15 in most of the groups although slight weight increases were observed. It could be deduced that nebulization had minimal weight gain effects compared to immunization.

Effects of first batch of treatment on nebulization (before sacrifice)

The early effects of the first batch of hydrocortisone (HC) and *E. hirta* (*EH*) treatment on the nebulization procedure could be evaluated on day 18. Treatment on day 18 was expected to alter the 'asthmatic' effects of nebulization.

Results from Table 2 and the graphs (Figures 3 and 4) indicate that animals treated with HC and *E. hirta* in both the short and long-term studies lost varying amounts of weight on day 18, most of which was statistically significant. However, the short and long-term control and asthma groups continued to gain relatively small amounts of weight on this day, indicating that treatment with HC remarkably reduced the cumulative weights gained following prior experimental procedures (immunization and nebulization), and in some cases to values approximate to those of Day 6. On the other hand, weights of the two *E. hirta* groups remained relatively similar to values on Day 15 in the LT study group but weights in the low *E. hirta* group during the LT study later reduced on Day 26 before increasing steadily along with weights in other groups.

All these results show that treatment with especially HC generally impacts negatively on the weight gain effects induced by other prior experimental procedures.

Midstream and late effects of continuous treatment

Treatment continued during days 22, 25, 29 and 32 of the long-term study and was suspended during days 34, 35 and 36 in order to repeat the nebulization procedure. The midterm effects of continuous treatment could be assessed from data obtained on day 26. Except for the low *EH* group, animals in most of the groups regained weight (albeit slightly) on this day after the plunge on day 18. The rate of weight gain was however more marked between days 26 and 32, indicating that a longer period of continuous treatment had remarkable cumulative effects on 'weight recovery'. Although treatment with HC was observed to cause a sharp weight loss on day 18, prolonged treatment appeared to initiate and sustain an increase in the rate of weight gain as observed between days 26 - 32.

Effects of repeated nebulization on weight changes

Effects of initial nebulization observed on Day 15 had minimal weight gain effects compared to immunization. Repeated immunization ended on day 36 of the long term study and effects of repeating this process were determined on Day 37. Results from Table 2 and the graphs show that weight increase was only very slight in almost all the test groups, *confirming the minimal effects of nebulization on weight gain.*

Early and terminal post-nebulization treatment effects of on weight changes

After nebulization, treatment resumed during days 39, 40, 41, 42, 43, 44, 45. Acute post-nebulization effects could be determined on day 42. Results from the line graphs show that weights increased sharply between day 37 and 42 instead of causing a sharp reduction following treatment after repeated nebulization. This effect was however not sustained after day 42 as animals in especially the two HC groups lost weight slightly while *EH* animals gained weight slightly. The reason for the weight disparity between the treatment groups on Day 46 is not clear but the weight loss effect observed in the HC-treated animals is consistent with previous findings (*Bernick & Zipkin, 1967*).

Comparison of progressive inter-group weight changes

Control versus asthma group

Throughout the study, percentage mean weights in the control groups were generally lower than in the asthma group as shown in Figures 3 and 4. The weight gain in the controls was at a relatively slower rate compared to other groups since animals in this group were not exposed to any procedure. The rate of weight gain in the asthma group was faster than in other groups at different periods during the study indicating that the effects of the different experimental procedures on weight change were more pronounced in the asthma group.

Control versus treatment groups

Percentage mean weights in all the treatment groups were higher than the control weights during most of the ST study period. During the LT study period however, weights in the two HC groups were lower than control weights from Day 26 following prolonged treatment. Weights remained relatively low until the end of the study albeit at about the same rate of weight change. The weight gain in the two *EH* groups were consistently higher than the controls throughout the study. These results suggest that long-term administration of HC causes a remarkably low but sustained weight gain pattern while prolonged administration of *EH* extracts causes only a slight but consistent weight gain.

Asthma versus other groups

The percentage weight values for the asthma group were higher than all other groups by Day 18. During the short-term study, the weights were lower than some of the groups and almost of the same value as others. Figures 3 and 4 shows that there is inconsistent weight change during the short-term period of the experiment but the weights in the asthma group were highest during the long-term study period.

The higher weight values in the asthma group and the consistent rate of weight gain in this group throughout the study indicate that immunization causes a high, steady and sustained rate of weight gain in mice, which can only be reduced by treatment. On the other hand challenge causes only a mild effect on weight change.

During the short-term study, administration of the low doses of both HC and *EH* appeared to be less effective in reducing weights to values below the weights in the asthma group but the rate of weight gain in the two *EH* groups appears to approximate that in the asthma group during the long-term period. This implies that treatment with *EH* causes less reduction in the weight gained due to induced asthmatic conditions, than treatment with HC. This finding is in line with a previous study using extracts of six medicinal plants *Cordyceps militaris* (CM), *Paecilomyces japonia* (PJ), *Phellinus linteus* (PL), *Ganoderma lucidum* (GL), *Grifola frondosa* (GF), and *Panax ginseng* (PG) in which body weight, weight gain, and FER (Food Efficiency Ratio) were found to have no significant effect on the in treated mice compared to the control group (*Jung et al, 2004*). Also, extracts of the Brazilian plant *Cissus sicyoides* were found to further reduce the weight loss caused by alloxan in diabetic animals (*Viana et al, 2004*) implying that *Cissus sicyoides* extracts are less effective in weight reduction.

In Figures 1 and 2, the lower the bars in the graph, the more effective is the group in reducing weight. It was found that the lower doses of the treatment agents used in this study were more effective than the higher doses. The reason for this is unclear but the “low dose of 100mg/kg” used in this study is only described in the context of a relative weight, when considering the higher dose of 125 mg/kg. In a previous study by Hausberger & Hausberger *(1958)* variable results were reported produced following a 5 mg/day cortisone dose.

Low dose versus high groups

During the short-term study period, there appeared to be no clear weight change pattern as seen in the long-term period. Weight gain was much higher in the "high dose groups" implying that the weights tended towards the values observed in the asthmatic group. The low dose weight data for both the low HC and low *EH* groups were however closer in value to the control group, indicating a low rate of weight gain compared to the corresponding high dose groups. As mentioned before, these results indicate that 100mg/kg body weight dose of HC and 25mg/kg dose of *EH* had a more effective impact on weight loss than the corresponding higher doses (125 mg/kg HC and 62.5 mg/kg *EH*) respectively.

Cortisone versus E. hirta groups

Weight patterns were generally irregular during the short-term period. However, the plant extract groups appeared to have higher weight values that closely approximated the asthma group for most of the long-term study duration. These results again showed that cortisone administration produces a lower rate of weight gain than does administration of *E. hirta* extracts, indicating that after the animals became asthmatic, the HC-treatment was more effective in restoring conditions to normal (near control) states than *EH*-treatment.

Conclusion

A guided stage-to-stage analysis of all data was undertaken to avoid excluding any possible contributions to weight change by the different experimental interventions, especially because causes of weight change are multifactorial. In this study, none of the animals lost weight below their starting (baseline) weights on the selected days and only at certain stages during the study (on days 18, 26 and 46) did animals lose weight relative to the weight values recorded on the previous study days.

Data obtained show that animals in both the ST and LT control groups experienced slow but steady progressive weight increments throughout the duration of the study. In addition, mean weights in all other groups also increased progressively in value.

It could be concluded that both immunization and nebulization had positive weight gain effects on the animals but the effects were more pronounced following immunization but were only minimal following nebulization. These effects were however modulated differently by treatment with the test agents (hydrocortisone and *E. hirta* extracts). It was notable that prolonged treatment with HC reduced the cumulative weight gained following prior experimental procedures (immunization and nebulization), followed by a slow and sustained increase in the rate of weight gain. On the other hand, prolonged administration of *EH* causes only a minimal reduction in weight gained due to induced asthmatic conditions. In addition, the lower doses were found to be more effective in lowering weights than the high doses.

The above conclusions are based on the assumption that the control group values represented animals with uninterrupted, normal, physiological states and the asthma group values represented the animals with "the asthmatic symptoms". Further studies with specific defined weight-related experimental goals would be required to clearly determine the possible effects of varying doses of especially the *Euphorbia hirta* plant extracts on animal weights, as well as to confirm the effects of hydrocortisone observed in this study.

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References

Aaron SD, D Fergusson, R Dent, Y Chen, K L Vandemheen, & R E Dales: Effect of weight reduction on respiratory function and airway reactivity in obese women. Chest 2004, *125*, 2046–2052.

Atallah AM & HJ Nicholas: Triterpenoids and steroids of *Euphorbia pilulifera* Phytochemistry 1972, *2*, 1860–1868.

Barnes BJ: Reactive oxygen species and airway inflammation. Free Radic Biol Med 1990, *9*, 235-43.

Beuther DA, ST Weiss & E R Sutherland: Pulmonary Perspective: Obesity and Asthma. Am J Respir Crit Care Med 2006, *174*, 112–119.

Bernick S & I Zipkin: Histochemical Study of Bone in Hydrocortisone- and Fluoride-Treated Rats. J Dent Res 1967, *46* (6), 1404-1411.

Bischof RJ, K Snibson, RJ Shaw & RJ Meeusen: Induction of allergic inflammation in the lungs of sensitized sheep after local challenge with house dust mite. Clinical and Experimental Allergy 2003, *33*, 367-375.

Blyth DI, TF Wharton, MS Pedrick, TJ Savage & S Sanjar: Airway subepithelial fibrosis in a murine model of atopic asthma: suppression by dexamethasone or anti-interleukin-5 antibody. Am J Respir Cell Mol Biol 2000, *23*, 241- 6.

Borovitskaya MP, NI Volodchenko, IB Vysokoostrovskaya & NA Zhukova: Side effects of hydrocortisone and prednisolone on mice of various age groups. Bulletin of Experimental Biology and Medicine 1971, *72* (6), 22–24.

Bwititi PT, T Machakaire, CFB Nhachi & CT Musabayane : Effects of *Opuntia megacantha* leaves extract on renal electrolyte and fluid handling in streptozotocin (STZ)-diabetic rats. Renal Failure 2001, *23*, 49–58.

Camargo CA Jr, ST Weiss, S Zhang, WC Willet & FE Speizer: Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. Arch Intern Med 1999, *159*, 2582–8.

Comalada M, D Camuesco, S Sierra, I Ballester, J Xaus, J Galvez & A Zarzuelo: In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappa B pathway. Eur J Immunol 2005, *35*, 584–592.

Curtis GH, MK Patrick, AG Catto-Smith & DG Gall: Intestinal anaphylaxis in the rat. Effect of chronic antigen exposure. Gastroenterology 1990, *98*, 1558 - 1566.

Dickshit RAO: Effect of Euphorbia hirta on the cardiovascular system.
Proc Indian Sci Con 1943, 349.

Dixon JB, L Chapman & P O'Brien: Marked improvement in asthma after Lap-Band surgery for morbid obesity. Obes Surg 1999, *9*, 385-9.

Dontas I, M Halabalaki, P Moutsatsou, S Mitakou, Z Papoutsi, L Khaldi, A Galanos & GP Lyritis: Protective effect of plant extract from *Onobrychis ebenoides* on ovariectomy-induced bone loss in rats. Maturitas 2006, *53*, 234242.

Epstein MM: Do mouse models of allergic asthma mimic clinical disease?
Int Arch Allergy Immunol 2004a, *133*, 84–100.

Epstein MM: Modeling allergic asthma: in vitro assays to virtual patients
Drug Discovery Today, Disease Models 2004b, *1*, 387-394

Epstein MM: Are mouse models of allergic asthma useful for testing novel therapeutics? Exp Toxicol Pathol 2006, *57*(2), 41-4 *Fenster CP, VM Darley-Usmar, AL Landar, BA Gower, RL Weinster, GR Hunter & RP Patel*: Weight loss and race modulate nitric oxide metabolism in overweight women. Free Radic. Biol. Med 2004, *37*, 695–702.

Galvez J, ME Crespo, J Jimenez, A Suarez & A Zarzuelo: Antidiarrhoeic activity of quercitrin in mice and rats. J Pharm Pharmacol 1993, *45*(2), 157-159.

Ganguli R: Weight gain associated with antipsychotic drugs. Journal of Clinical Psychiatry 1999, *60* (21):, 20–24.

Gleich GJ, NA Flavahan, T Fujisawa & PM Vanhoutte: The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity. J Allergy Clin Immunol 1988, *81*, 776-81.

Goudie AJ, J Smith & J Halford: Characterization of olanzapine-induced weight gain in rats.

Journal of Psychopharmacology 2002, *16*, 291–296.

Gupta DR & SK Garg: A chemical examination of *Euphorbia hirta* Linn. Bull Chem Soc Jpn 1966, *39* (11), 2532–2534.

Hakala K, B Stenius-Aarniala & A Sovijarvi: Effects of weight loss on peak flow variability, airways obstruction, and lung volumes in obese patients with asthma. Chest 2000, *118*, 1315–1321.

Harris RB, J Zhou, BD Youngblood, II Rybkin, GN Smagin & DH Ryan: Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets. Am J Physiol 1998, *275* (6, 2), 1928–38.

Hausberger FX & BC Hausberger: Effect of insulin and cortisone on weight gain, protein and fat content of rats. Am J Physiol 1958, *193*, 455 - 460.

Hazleton LW & RC Hellerman: Studies on the pharmacology of *E pilulifera*. J Am Pharmal Assoc 1954, *40*, 474-476.

Huneau JF, M Coste & D Tome: Effect of chronic antigen exposure in growth and intestinal histamine content of sensitized rats. Gastroenterologie Clinique et Biologique 1991, *15*, 525-528.

Hyman M: Ultrametabolism. Simon and Schuster (Scribner) Inc: New York. 2006

Johnson JB, W Summer, RG Cutler, B Martin, DH Hyun, VD Dixit, M Pearson, M Nassar, R Tellejohan, S Maudsley, O Carlson, S John, DR Laub & MP Mattson: Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. Free Radical Biology and Medicine 2007, *42*, 665–674.

Jung K, IH Kim & D Han: Effect of medicinal plant extracts on forced swimming capacity in mice. Journal of Ethnopharmacology 2004, *93* (1), 75-81.

Jungsuwadee P, G Dekanb, G Stingla & MM Epstein: Inhaled dexamethasone differentially attenuates disease relapse and established allergic asthma in mice. Clinical Immunology 2004, *110*, 13-21.

Kandaswami C, E Middleton: Free radical scavaging and antioxidant activity of plant flavonoids. Adv Exp Med Biol 1994, *366*, 351–376.

Kharitinov SA, D Yates, RA Robbins, R Logan-Sinclacir, EA Shinebourne, PJ Barnes: Increased nitric oxide in exhaled air of asthmatic patients. Lancet 1994, *342*,133-5.

Kokwaro JO: Medicinal plants in East Africa East African Literature. Bureau Nairobi 1976, 1-25.

Kumar A, RL Sorkness, MR Kaplan, RF Lemanske Jr: Chronic, episodic, reversible airway obstruction after viral bronchiolitis in rats. Am. J Respir Crit Care Med 1997, *155,*130-4.

Kumar RK & PS Foster: Modeling allergic asthma in mice; pitfalls and opportunities. Am. J. Respir Cell Mol Biol 2002, *27*, 267-272.

Jung K, IH Kim & D Han: Effect of medicinal plant extracts on forced swimming capacity in mice. Journal of Ethnopharmacology 2004, *93*(1), 75-81.

Landstra AM, HM Boezen, DS Postma & WM Van Aalderen: Effect of intravenous hydrocortisone on nocturnal airflow limitation in childhood asthma. Eur Respir J 2003, *21*, 627–632.

Lanhers MC, J Fleurentin, P Dorfman, F Mortier & JM Pelt: Analgesic antipyretic and anti-inflammatory properties of *Euphorbia hirta.* Planta Med 1991, *573*, 225-231.

Leigh R, R Ellis, J Wattie, DS Southam, M de Hoogh, J Gauldie, PM O'Byrne & MD Inman: Dysfunction and remodeling of the mouse airway persist after resolution of acute allergen-induced airway inflammation. Am J Respir Cell Mol Biol 2002, *27*, 526-35.

Le Strange R: A history of herbal plants Angus and Robertson. London;1977, 1-50.

Macgregor AM & RA Greenberg: Effect of surgically induced weight loss on asthma in the morbidly obese. Obes Surg 1993, *3*,15-21.

Marti O, J Marti & A Armario: Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. Physiol Behav 1994, *55*, 747-753.

Martinez-Vazquez M, TOR Apan, ME Lazcano & R Bye R: Anti-inflammatory active compounds from the n-hexane extract of Euphorbia hirta. Revista de la Sociedad Quimica de Mexico 1999, *443* (4), 103-105.

McDermott, R, F Tulip, B Schmidt & A Sinha: Sustaining better diabetes care in remote indigenous Australian communities. British Medical Journal 2003, *327*, 428-430.

McMillan SJ & CM Lloyd: Prolonged allergen challenge in mice leads to persistent airway remodeling. Clin Exp Allergy 2004, *34*, 497-507.

Musabayane CT, N Mahlalela, FO Shode & JAO Ojewole: Effects of *Syzygium cordatum* (Hochst) [Myrtaceae] leaf extract on plasma glucose and hepatic glycogen in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 2005, *97*, 485-490.

Nadeem A, SK Chhabra, A Masood & HG Raj: Therapeutic augmentation of the antioxidant defenses might be beneficial. J Allergy Clin Immunol 2003, *111*,72-8.

Nagao K, H Akabane, T Masuda, M Komai, H Tanaka & H NagaI: Effect of MX-68 on airway inflammation and hyper-responsiveness in mice and guinea pigs. J Pharm Pharmacol 2004, *56*(2),187-96.

Oliver B: Medicinal Plants in Nigeria (first edition). College of Arts Science and Technology Ibadan Nigeria 1959, 138-149.

Ottenweller JE, RJ Servatius, WN Tapp, SD Drastal, MT Bergen & BH Natelson: A chronic state in rats: Effects of repeated stress on basal corticosterone and behaviour. Physiol Behav 1992, *51*, 689–698.

Park SJ & YC Lee: Antioxidants as Novel Agents for Asthma. Mini Reviews in Medicinal Chemistry 2006, *6* (2), 235-240(6).

Pretorius E, OE Ekpo, E Smit: Comparative ultrastructural analyses platelets and fibrin networks using the murine model of asthma. Experimental and Toxicologic Pathology 2007, *59* (2), 105-114.

Retana-Márquez S, H Bonilla-Jaime, G Vázquez-Palacios, E Domi´nquez-Salazar, R Marti´nez-Garcia & J Velázguez-Moctezuma: Body weight gain and diurnal differences of corticosterone changes in response to acute and chronic stress in rats. Psychoneuroendocrinology 2003, *28*, 207–227.

Saldanha JCS, DL Gargiulo, SS Silva, FH Carmo-Ponto, MC Andrade, JI Alvarez-Leite, MM Teixera & DC Cara: A model of chronic IgE-mediated food allergy in ovalbumin-sensitized mice. Braz J Med Biol Res 2004, *37* (6), 809-816.

Shahana S, E Björnsson, D Ludviksdottir, C Jansson, O Nettelbladt, P Venge & GM Roomans: Ultrastructure of bronchial biopsies from patients with atopic and non-atopic asthma. Am. J. Respir. Crit. Care Med 2003, *167*, 204.

Shebani E, S Shahana, C Janson, GM Roomansa, on behalf of the BHR group: Attachment of columnar airway epithelial cells in asthma. Tissue and Cell 2005, *37*,145–152.

Shore SA: Obesity and asthma: cause for concern. Current Opinion in Pharmacology 2006, *6* (3), 230-236.

Snibson KJ, RJ Bischof, RF Slocombe & ENT Meeusen: Airway remodeling and inflammation in sheep lungs after chronic airway challenge with house dust mite. Clin Exp Allergy 2005, *35*,146-152.

Sofowora A: Medical Plants and Traditional Medicine in Africa. First Edition, Chichester UK: John Wiley and Sons,1984, 114-213.

Stenius-Aarniala B, T Poussa, J Kvarnstrom, EL Gronlund, M Ylikahri & P Mustajoki: Immediate and long term effects of weight reduction in obese people with asthma: randomised controlled study. Brit Med Jnl 2000, *320***,** 827-832.

Viana GSB, ACC Medeiros, AMR Lacerda, LKAM Leal, TG Vale & F Jose de a Matos: Hypoglycemic and anti-lipemic effects of the aqueous extract from *Cissus sicyoides*. BMC Pharmacology 2004, *4*, 9

Watt MJ & MG Breyer-Brandwijk: The Medicinal and poisonous plants of Southern and Eastern Africa, 2nd Edition London. E and S Livingstone, 1962, 408-410.

Weiss ST & S Shore: Obesity and asthma: directions for research. Am J Respir Crit Care Med 2004,*169*, 963–968.

Wong TF: Medicinal plants of Mauritius. E NDA Publications 10; Dakar Senegal. 1980

Zhao GD, A Yokoyama, N Kohno, K Sakai, H Hamada & K Hiwada K: Effect of suplatast tosilate (IPD-1151T) on a mouse model of asthma: inhibition of eosinophilic inflammation and bronchial hyperresponsiveness. International Archives of Allergy and Immunology 2000, *121* (2), 116-22.