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Novel Weight-Reducing Activity of *Galega officinalis* in Mice

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Abstract

Galega officinalis (galega, Goat's Rue, French Lilac) is well known for its hypoglycaemic action and has been used as part of a plant mixture in the treatment of diabetes mellitus. During pharmacological investigations of an ethanolic extract of a powdered mixture of equal proportions of *G. officinalis*, *Cressa cretica*, *Mangifera indica* and *Syzygium jambolanum*, a weight reducing effect of galega was discovered. In this study we have investigated the novel weight reducing effect of galega in mice.

Galega herb (10% w/w in the diet) caused a significant reduction in body weight in both normal and genetically obese (ob/ob) animals treated for 28 days when compared with respective controls ($P < 0{\cdot}01$). In normal mice, the weight loss was reversible and initially associated with a transient reduction in food intake but was then maintained even in the presence of increased eating above the control level. Pair-fed normal mice receiving galega for seven days also showed significant weight loss ($P < 0{\cdot}01$, compared with the control) in the presence of increasing food intake. In sharp contrast, weight loss in galega-treated ob/ob mice was accompanied by a persistent reduction in food intake over the 28-day treatment period. Post-mortem examinations of all galega-treated mice revealed a striking absence of body fat. Serum glucose was significantly reduced in both strains of mice receiving galega for 28 days ($P < 0{\cdot}01$), whereas serum insulin was significantly reduced only in obese mice ($P < 0{\cdot}01$).

In summary, together with its established hypoglycaemic effects, galega has a novel weight reducing action that, in normal mice, is largely independent of a reduction in food intake. The mechanism of the weight reducing action of galega is unclear but involves loss of body fat.

Galega (*Galega officinalis*, Goat's Rue, French Lilac) was used as a treatment for diabetes in Medieval Europe (Oubre et al 1997). There is at least one active hypoglycaemic agent in the plant, the guanidine derivative galegine, which was isolated by Tanret (1914). Galegine is present in the aerial parts of the plant (Reuter 1962, 1964; Schreiber et al 1962) and although it is too toxic for clinical use, its discovery led to the development of metformin, a biguanide which has been used to treat non-insulin dependent diabetes mellitus for the past four decades (Oubre et al 1997; Day 1999). Ethanolic extracts of a powdered mixture of equal proportions of *G. officinalis*, *Cressa cretica*, *Mangifera indica* and *Syzygium jambolanum* have been used in the treatment of patients with diabetes mellitus (Anand 1989, personal communication). It was during the pharmacological investigation of this treatment that the novel weight reducing activity of powdered galega herb was discovered (Palit et al 1996, 1998a). In this study we have investigated its weight reducing effect in normal and genetically obese mice.

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Materials and Methods

Animals

Adult male mice (BKA) were obtained from B & K Universal Ltd (Grimston, Hull, UK) and genetically obese female mice (C57BL/6-ob/ob/Ola/Hsd) from Harlan Olac Ltd (Blackthorn, Oxon, UK). Mice were housed in an environment maintained at $21 \pm 2^{\circ}$C, with humidity of $55 \pm 10\%$ and a 12-h light–dark cycle (lights on at 0600 h). Mice were

supplied with a standard pellet diet (CRM, Special Diet Services, Manea, Cambridge, UK) and tap water was freely available.

Plant material

Galega (dried aerial parts, coarsely ground, Batch No. 2123) was obtained from Brome & Schimmer Ltd (Hampshire, UK) through Dr C. L. Anand (Natural Medicines Research Trust, Glasgow, UK). The plant material had been collected during the flowering period and was authenticated by Lesley Harris (National Institute of Medical Herbalists).

^{1}H NMR 400·14 MHz and ^{1}H-^{1}H-cosy-45 spectroscopic analysis of an ethanol–water extract of the powdered galega revealed the presence of glycose-type compounds, aromatic-type compounds and there was also evidence of galegine. TLC of the extract also confirmed the presence of galegine (R_f values for the extract and for pure galegine were 0·67 and 0·68, respectively) (Palit et al 1998b)). The galegine content of galega is highest during florescence (Schaefer & Stein 1969), which is when the plant material had been collected.

Diet preparation

For the purposes of this study, the plant material was firstly ground into a fine powder. It was then incorporated into the standard feed of mice at 1·25, 2·5, 6·25 or 10% w/w, by mixing with powdered CRM diet. Finally, the galega-containing diet was re-pelleted before being supplied to mice. Control diet was prepared similarly but without the addition of plant material.

Measurements

Blood samples for determination of serum glucose and insulin were collected from the trunk following stunning and decapitation. Serum glucose was determined using a Beckman glucose analyser (Beckman Instruments Inc., Fullerton) and serum insulin was measured by radioimmunoassay, based on a standard kit method (^{125}I insulin kit, ICN Biomedicals Inc., Costa Mesa, USA). Body weight and food intake were measured using an electronic balance with a tolerance of $\pm 0{\cdot}01$ g and water intake was determined using a measuring cylinder. Urine volume was measured over 1 h by placing the mice in metabolic cages, which allowed collection of faeces-free urine. The volume of urine produced was accurately measured by drawing it into a 1·0-mL syringe.

Study on dose-related effects in normal mice

This study investigated dose-related effects of galega on a number of physiological parameters in normal mice. They also allowed determination of a suitable concentration of galega to use in subsequent studies. Groups of 12–13 normal mice were randomly allocated to receive control diet or treated diet (containing 1·25, 2·5, 6·25 or 10% w/w galega), freely for seven 7 days. Body weight, food and water intake were monitored daily. Blood samples for determination of serum glucose were collected at the end of the treatment period.

Study comparing effects in normal and genetically obese mice

This study investigated the effects of galega on a number of physiological parameters in normal and ob/ob mice. Groups of six to eight mice were randomly allocated to receive control or treated diet (containing 10% w/w galega), freely for 28 days. Body weight, food and water intake were monitored daily. Blood samples for determination of serum glucose and insulin were collected at the end of the treatment period.

Pair-feeding study in normal mice

The pair-feeding study determined if the weight reducing activity of galega was due to a reduction in food intake. Thus, 18 mice were randomly allocated to receive control or treated diet (containing 10% w/w galega). They were then divided into nine pairs matched for body weight. Initially (day 0), all mice received control diet freely. On day 1 and subsequently, one member of each pair received galega-containing diet freely instead. The other mouse of each pair continued to receive control diet freely until day 2. From day 2 onwards, the amount of diet offered to controls each day was equal to the amount of galega-containing diet consumed on the previous day by its treated-pair partner. Body weight and food intake were monitored daily and after seven days blood samples were collected for determination of serum glucose.

Reversibility study in normal mice

This study investigated the reversibility of the weight reducing effects of galega and involved two groups of normal mice ($n = 6$ in each). One group was randomly allocated to receive control diet for the entire duration of the study. The other group received treated diet (containing 10% w/w galega) for a fortnight, followed by a change to control diet from day 15 onwards. Mice were allowed to feed on their diet freely. Body weight, food and water intakes were monitored initially on days 0, 1, 4, 8,

11 and then daily. After 28 days, blood samples were collected for determination of serum glucose.

Study on effects on urine volume in normal mice

This study determined the effects of galega on urine volume. Normal mice were randomly allocated to receive either control diet or diet containing galega (10% w/w), freely for seven days. Body weight, food and water intakes were monitored daily. On day 7, urine volume produced over 1 h was determined in control and treated animals ($n = 5$, randomly chosen from each group).

Statistical analysis

Groups of data for body weight, urine volume and serum glucose were expressed as mean ± s.e.m. Serum insulin values were expressed as geometric mean with 95% confidence limits. In the pair-feeding study, it was necessary to house mice individually, and therefore food and water intakes were expressed as mean ± s.e.m. In all other studies, mice in one group were housed together in a single cage. Therefore, in these instances food intake for each group was expressed as a daily average, which was calculated by weighing the reduction in food weight over 24 h and dividing this value by the number of mice in the cage. Average daily doses of galega could then be determined by taking into account the percentage of galega in the diet. Mean daily dose of galega (± s.e.m.) over a given treatment period could also be calculated using daily average values. Water intake was determined by measuring the reduction in water volume over 24 h and dividing this value by the number of mice in the cage, thus giving average daily water intake.

Data were compared using Student's unpaired *t*-test, two-way analysis of variance or two-way analysis of variance for repeated measures, as appropriate. Differences were considered to be significant if $P \leq 0{\cdot}01$.

Results

Study on dose-related effects in normal mice

The mean daily doses of galega consumed over the treatment period increased with rising dietary concentration of the herb (1·25, 2·5, 6·25 and 10% w/w), and were as follows: $2{\cdot}1 \pm 0{\cdot}04$, $3{\cdot}7 \pm 0{\cdot}2$, $7{\cdot}8 \pm 0{\cdot}9$ and $13{\cdot}7 \pm 2{\cdot}7\ g\,kg^{-1}$, respectively. Galega produced a temporary dose-related reduction in food intake, e.g. on day 1: 22, 43, 67 and 78% reduction compared with control, at mean daily doses of 2·1, 3·7, 7·8 and $13{\cdot}7\ g\,kg^{-1}$, respectively. Only treatment with the higher doses of galega i.e. 7·8 and $13{\cdot}7\ g\,kg^{-1}$ produced significant and sustained reduction in body weight ($P < 0{\cdot}01$) and significant lowering of serum glucose ($P < 0{\cdot}01$), compared with the control.

Study comparing effects in normal and genetically obese mice

Galega (10% w/w in the diet) caused a significant and time-dependent reduction in body weight in both normal and ob/ob mice (Figures 1A, 2A, respectively, $P < 0{\cdot}01$). In normal mice, over the first five days food intake in the treated group was generally lower than the control but was showing an upward trend. From day 6 onwards it was always above, and approximately double the control values towards the end of the treatment periods (Figure 1B). In ob/ob mice, food intake in the treated group was lower than the control at all times (Figure 2B).

Galega caused a significant decrease in serum glucose in both normal and ob/ob mice when compared with the respective controls (normal

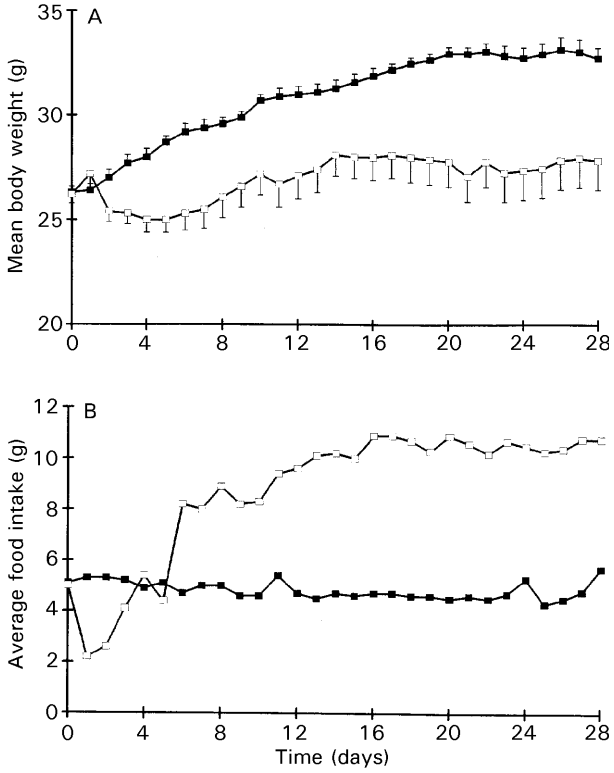

Figure 1. Effect of galega on body weight and food intake of normal mice ($n = 7-8$). A. Mean daily body weight: effects of treatment, time, interaction $P < 0{\cdot}01$; two-way analysis of variance for repeated measures. Values are mean (± s.e.m.). B. Average daily food intake. ■ Control, □ treated.

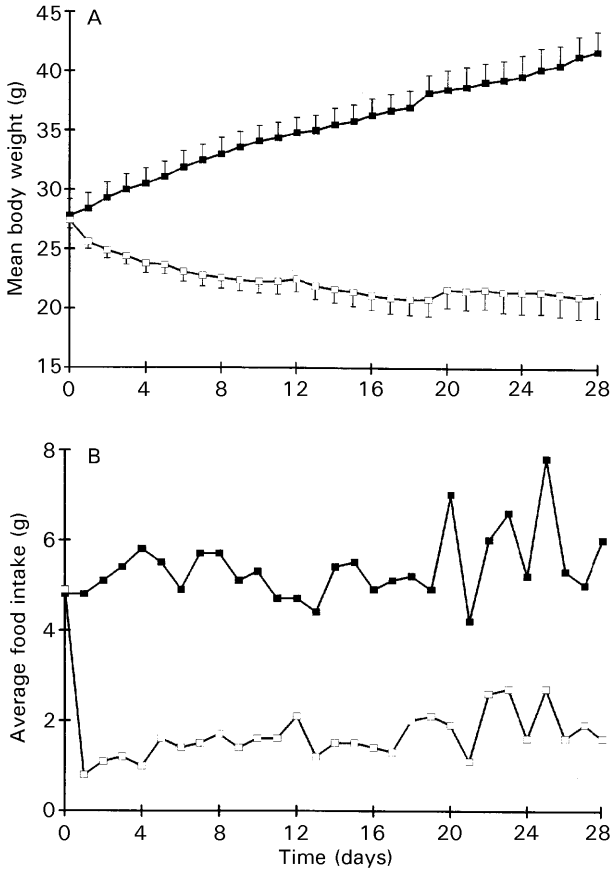

Figure 2. Effect of galega on body weight and food intake of ob/ob mice (n = 6–7). A. Mean daily body weight: effects of treatment, time, interaction $P < 0.01$; two-way analysis of variance for repeated measures. Values are mean ± s.e.m. B. Average daily food intake. ■ Control, □ treated.

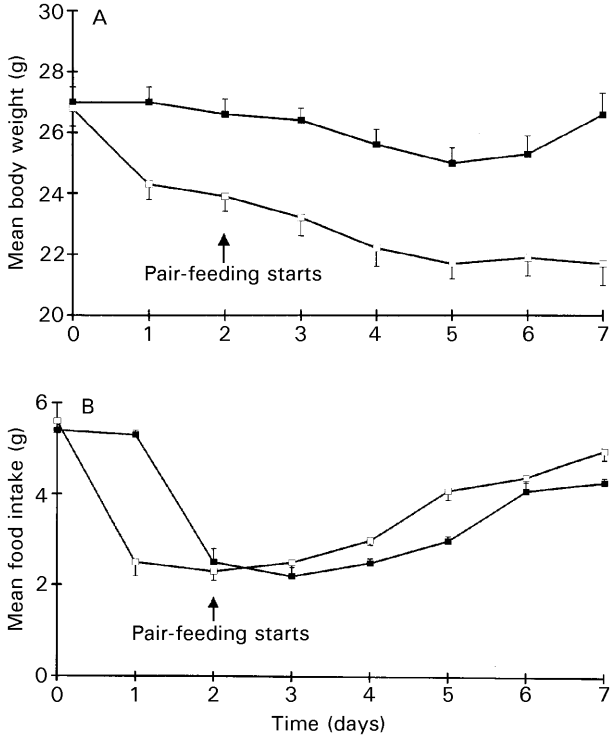

Figure 3. Effect of galega on body weight and food intake in pair-fed normal mice (n = 9). A. Mean daily body weight: effects of treatment, time, interaction $P < 0.01$; two-way analysis of variance for repeated measures. Values are mean ± s.e.m. B. Mean daily food intake. ■ control, □ treated.

mice: 7.0 ± 0.4 vs 9.8 ± 0.5 mmol L, $P < 0.01$; obese mice: 8.5 ± 1.2 vs 13.9 ± 0.6 mmol L^{-1}, $P < 0.01$). It also produced a significant reduction in serum insulin in ob/ob mice (6·4 (6·1, 6·9) vs 10·5 (9·4, 11·8) ng mL^{-1}, $P < 0.01$). However, serum insulin in galega-treated normal mice was slightly but not significantly reduced when compared with the control.

Pair-feeding study in normal mice

Mice treated with galega (10% w/w in the diet) showed a significant decrease in daily body weight compared with the control (Figure 3A, $P < 0.01$). Over the first four days of treatment with galega, this weight loss was accompanied by food intake that was much lower than the freely fed control intake of day 1 (Figure 3B). From day 4, a reduced body weight of approximately 22 g was maintained until the end of the treatment period, in spite of a rapidly increasing food intake. In contrast, control mice showed a gradual increase in body weight over days 5–7 (Figure 3A) as food intake increased (Figure 3B). At the end of the study, serum glucose in galega-treated mice was significantly lower than in the controls (6.2 ± 0.2 vs 9.4 ± 0.5 mmol L^{-1}, $P < 0.01$).

Reversibility study in normal mice

Treatment with galega (10% w/w in the diet) caused a significant and time-dependent reduction in body weight over days 0–15, compared with the control (Figure 4A, $P < 0.01$). However, within 24 h of stopping treatment with galega there was a significant and time-dependent increase in body weight. Moreover, by the end of the study, body weight had almost reached the control value and was still showing an upward trend (Figure 4A, days 15–28, $P < 0.01$).

At the start of the study, food intake in the treated group was initially reduced and then gradually increased to above control intake by day 14. However, within 24 h of stopping treatment with galega, food intake decreased and then remained similar to the control group for the rest of the study (Figure 4B). At the end of the study, there was no significant difference between the mean serum glucose levels of the control group and the group that had received galega for a fortnight.

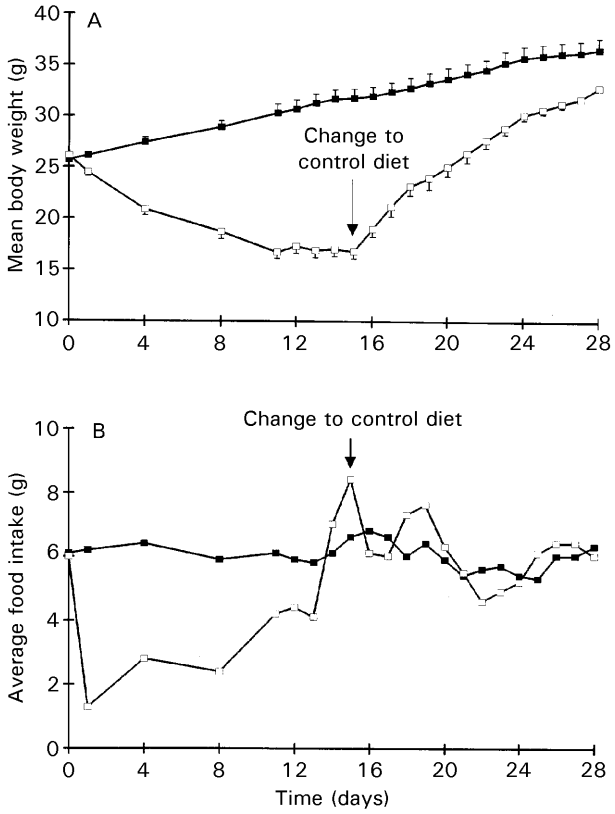

Figure 4. Reversible effects of galega on body weight and food intake of normal mice (n = 6). A. Mean daily body weight: effects of treatment, time, interaction (days 0–15) $P < 0{\cdot}01$; effects of stopping treatment, time, interaction (days 15–28) $P < 0{\cdot}01$; two-way analysis of variance for repeated measures. Values are mean ± s.e.m. B. Average daily food intake. ■ Control, □ treated.

Study on effects on urine volume in normal mice

Mice treated with galega (10% w/w in the diet) for seven days, showed a slight but non-significant increase in urinary output compared with the control ($0{\cdot}35 \pm 0{\cdot}04$ vs $0{\cdot}50 \pm 0{\cdot}04$ mL h^{-1}/100 g).

General observations

In all of the above studies, galega-treated mice appeared to be no different in terms of their behaviour and general health when compared with the controls. The treated mice showed normal appearance, grooming and activity, and there were no signs of diarrhoea or steatorrhoea. Post-mortem examination of normal and ob/ob mice receiving galega (10% w/w in the diet) until they were killed, showed a striking loss of general body fat and the complete absence of epididymal fat pads (abdominal fat) when compared with the respective controls.

Discussion

Galega produced a significant reduction in serum glucose in freely fed normal and ob/ob mice, receiving the herb at a dietary concentration of 10% w/w for 28 days. At this concentration, galega also significantly reduced serum insulin in freely fed ob/ob mice. The hypoglycaemic action of galega has been previously demonstrated in both normal and diabetic animals including mice (Shukyurov et al 1974; Petricic & Kalodera 1982) and can be attributed to at least one agent, galegine, a guanidine derivative present in the aerial parts of the plant (Petricic & Kalodera 1982). However, the weight reducing action of galega is a novel observation that has not been reported previously. The weight-reducing and hypoglycaemic effects are clearly dose-related. They occurred to a significant extent only at higher doses of galega obtained through dietary concentrations of 6·25 and 10% w/w of the herb. The latter concentration was used in all subsequent studies, since any toxicity was more likely to manifest at the higher dose.

The studies on comparative effects of galega in normal and ob/ob mice showed that galega caused a sustained and significant reduction in body weight in both strains of mouse. In normal mice, over the first five days, weight loss was associated with a transient reduction in food intake. However, subsequently weight reduction was maintained even when the mice began eating normally and indeed even when their food intake was approximately double that of the control. Furthermore, in the pair-feeding study, although galega-treated and control mice showed weight loss when food intake was reduced, only controls regained weight as food intake increased. These studies clearly indicated that in normal mice, the weight reducing effect of galega was considerably independent of a reduction in food intake. *Galega officinalis* appears to have some nutritional value since its protein quality has been shown to be as good as that of the fodder crop lucerne and is greater in quantity (Isajev et al 1954). Thus, it is unlikely that the increase in food intake observed in normal mice was simply linked to a reduced nutritive value of their diet due to incorporation of plant material. Furthermore, towards the end of the 28-day study, treated animals were consuming about twice as much as the control. This increase is far in excess of that which would be required to compensate for a 10% reduction in nutritional value of the diet. Galega has been shown to have inhibitory effects on intestinal glucose transport (Neef et al 1996). This finding may provide some evidence that the weight-reducing effect of galega in normal mice during increased eating might be partly due to a nutritive deficiency of glucose, arising from reduced intestinal absorption of this nutrient. The causes and consequences of increased food intake in galega-treated normal mice require further investigation.

In contrast, in galega-treated ob/ob mice, weight loss was accompanied by a persistent reduction in food intake. Thus, although galega was weight-reducing in both normal and ob/ob mice, the observed effects on food intake were markedly different in the two strains of mice. This would suggest that there are differences in the mechanism of the weight reducing action of galega in normal and ob/ob mice. It is possible that the initial reduction in food intake in normal mice and the sustained reduction observed in ob/ob mice was at least partly due to a bitter tasting substance called peganine, present in the aerial parts of the herb (Schreiber et al 1962; Schaefer & Stein 1967; Koehler 1969). Obese mice may have been more sensitive than normal mice to the effects of peganine, resulting in a sustained reduction in food intake only in the ob/ob strain.

More studies are needed to test all of the above hypotheses and to elucidate the exact mechanism of the weight reducing action of galega in normal and ob/ob mice. Whatever this may be, it involves the loss of body fat as was suggested by the post-mortem observations in galega-treated mice of both strains. Furthermore, galegine has been shown to inhibit glucose oxidation and lipogenesis in-vitro (Hoppe-Seylers 1971) and these findings require further investigation in relation to the weight reducing activity of galega. The reputed diuretic effect of galega (British Herbal Pharmacopoeia 1976) is unlikely to make a major contribution to its weight-reducing effect, since in the present studies at least, galega-treated normal animals did not show a significant increase in urinary output compared with the control.

In the reversibility study in normal mice, the weight reducing effect of galega was again similar to that observed in other studies, i.e. a sustained weight reduction initially associated with a decrease in food intake but then maintained in the presence of increasing and increased eating above control levels. However, on placing the galega-treated mice onto the control diet there was a rapid regain of weight as food intake returned to normal. These results showed that the weight reducing action of galega and its effect on food intake were reversible. In view of the hypoglycaemic effect of galega in normal mice observed in other studies, the absence of a significant difference in serum glucose between the treated and control group in this study, suggests that the hypoglycaemic action of galega is also reversible.

The toxicity of *Galega officinalis* in various animal species is well documented. For example, toxicosis has been observed in ewes after ingestion of the plant (Faliu et al 1982; Keeler et al 1986). Petricic & Kalodera (1982) reported an acute oral LD50 in mice (strain not specified) at a dose equivalent to $30\,g\,kg^{-1}$ of dried galega herb. Although this LD50 value falls within the range of average daily doses of galega consumed by normal mice in this study, i.e. $8–40\,g\,kg^{-1}$ using 10% w/w dietary galega, there were no overt signs of toxicity as judged subjectively by the behaviour and general health of the treated animals compared with controls. Similarly, there were no signs of toxicity in ob/ob mice in which average daily doses of galega were between $3–13\,g\,kg^{-1}$. Of course, the average doses consumed by ob/ob mice were much lower than the LD50 dose reported by Petricic & Kalodera (1982). A possible explanation of the observed lack of toxicity of galega in this study, is that on chronic administration of the herb, mice developed tolerance to any toxic effects. In fact, apparent adaptation to the toxin of *G. officinalis* has been reported in ewes (Keeler et al 1986). It is also possible that any toxic effects of galega were asymptomatic or that it is not toxic in the strains of mice used. Another explanation might relate to the amounts of toxic compounds present in galega. The toxins are known to be galegine, hydroxygalegine and peganine, and the plant exhibits a marked variability in the content of these compounds, both within species as well as within populations (Schaefer & Stein 1967, 1969). The batch of galega used in this study may only have contained relatively small amounts of these toxic agents, meaning that the doses of toxins consumed by the treated mice were too low to produce overt toxicity. Further investigations into the toxicity of galega in mice are needed in order to test these hypotheses.

To conclude, in both normal and ob/ob mice, galega has novel weight reducing activity which is accompanied by its well known hypoglycaemic effect. The extent to which these activities are independent of each other should be determined and the active compound(s) responsible need to be isolated and identified. In view of the different effects of galega on food intake in normal and ob/ob mice, it is probable that the mechanism of the weight reducing action differs between these strains. The exact nature of this mechanism(s) needs to be investigated but involves loss of body fat, and in normal mice is clearly partly independent of a reduction in food intake. Although there were no apparent toxic effects of galega in either strain of mouse, this finding requires further investigation, especially in view of the documented toxicity of the herb in various mammalian species including mice.

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