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Excess use of *Momordica charantia* extract may not be safe with respect to thyroid function and lipid peroxidation

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Effects of alcoholic extract of *Momordica charantia* fruits (100, 200, 400 and 500 mg kg^{-1} body weight day^{-1} for 15 days) on the alterations in serum thyroxine (T$_4$) and triiodothyronine (T$_3$) concentrations and on hepatic lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activities were studied in adult male mice. Higher concentrations of *M. charantia* (400 and 500 mg kg^{-1}) decreased the serum concentrations of T$_3$ and T$_4$ and enhanced hepatic LPO with a concomitant decrease in CAT activities, indicating a thyroid inhibitory and peroxidative role of the plant extract. However, with the lower doses (100 and 200 mg kg^{-1}) these adverse effects were not seen. Although 200 mg kg^{-1} was found to enhance T$_3$, T$_4$ was reduced. Since two higher doses inhibited thyroid hormone concentrations and increased hepatic LPO, we suggest that *M. charantia* fruit extract, when used in excess may prove to be harmful with respect to thyroid function and lipid peroxidation.

BITTERGOURD, *Momordica charantia*, Linn (Family-Cucurbitaceae), commonly known as 'Karela' in India is a climbing plant, cultivated throughout southern Asia. This plant is mainly used for the consumption of its fruits as vegetables. While, its antispermatogenic, hypoglycaemic and antidiabetic properties have been documented from time to time[1-3], no study was made with respect to alterations in thyroid hormone(s). In fact, literature on the regulatory role of commonly used consumable plant materials on the thyroid function is meagre[4-6], despite the fact that thyroid gland is one of the most important endocrine organs[7], primarily responsible for the regulation of body metabolism. Therefore, in the present study, an attempt has been made to reveal the possible role of *M. charantia* fruit extract in the alteration of two thyroid hormones, triiodothyronine (T$_3$) and thyroxine (T$_4$). Lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activities were also investigated in order to evaluate the safe nature of the extract.

Diethylene triamine pentaacetic acid (DTPA) was purchased from Sigma Chemical Co, USA and thiobarbituric acid (TBA) was from E. Merck, Germany. Sodium dodecyl sulphate (SDS), pyrogallol and hydrogen peroxide were of reagent grade, obtained from Loba Chemie, Mumbai, India. Radioimmunoassay (RIA) kits for the quantification of T$_4$ and T$_3$ levels were supplied by Bhabha Atomic Research Centre (BARC), Mumbai, India.

An alcoholic extract of Momordica fruits was prepared according to the method of Shibib *et al.*[3]. In brief, fresh fruits purchased from the local market were thoroughly washed in tap water, cut into pieces and the seeds removed manually. About 1 kg of seedless vegetable was then blended with 1500 ml of 95% ethanol, left at room temperature with occasional shaking for 48 h. The suspension was filtered through a cheesecloth and the filtrate was evaporated at 40–50°C to remove alcohol and to produce a free flowing powder. On the day of experimentation, the desired amount of powder was suspended in distilled water for the final administration.

Three-month-old Swiss albino male mice weighing 32 ± 3 g were used in five groups of 8 each. Groups II, III, IV and V were administered with different doses (100, 200, 400 and 500 mg kg^{-1} body wt) of *M. charantia* extract by gastric incubation. Two of these doses (200 and 500 mg kg^{-1}) were taken from earlier studies[2,3]. The other two doses were considered because of the fact that our preliminary investigation with 500 mg kg^{-1} proved to be toxic (data not shown). Group I, receiving 0.1 ml of vehicle (distilled water) served as control. The treatment was continued for 15 days. On the last day of experimentation, blood was collected from each animal and serum was separated by centrifugation and stored at –20°C for the estimation of T$_3$ and T$_4$ levels. After exsanguination, the liver was removed, washed twice in phosphate buffered saline and immediately processed for estimation.

Serum concentrations of T$_3$ and T$_4$ were estimated by RIA, following the protocol of BARC, as followed earlier in our laboratory[8,9]. Lower limits of sensitivity for T$_3$ and T$_4$ were 0.07 ng ml^{-1} and 0.12 ng ml^{-1}, respec-

tively. Inter-assay variation was less than 5% for both the hormones.

Liver was homogenized in 10% (w/v) ice-cold 0.15 M phosphate buffer (pH 7.4) using Potter–Elvehjem teflon homogenizer and the homogenate was centrifuged at 15,000 g at 4°C for 30 min. Assay of LPO was done by the method of Ohkawa et al.[10] and the amount of malondialdehyde (MDA) formed was measured by taking the absorbance at 532 nm (extinction coefficient 1.52×10^5) using Shimadzu UV-160 A spectrophotometer. LPO was finally expressed as the nmole of MDA formed per h per mg protein.

Hepatic SOD activity was assayed according to the method of Marklund and Marklund[11], and the enzyme activity was expressed as units per mg protein. One unit of this enzyme is defined as the enzyme activity that inhibits autooxidation of pyrogallol by 50%. CAT activity was estimated in the liver homogenate following the

method of Aebi[12], which was expressed as μmol H_2O_2 decomposed per min per mg protein. Hepatic protein content was estimated according to the method of Lowry et al.[13]. For statistical evaluation of data, analysis of variance (ANOVA) followed by Student's 't' test was used.

The results are summarized in Figure 1 and Table 1. Dose-specific alterations in hepatic LPO and in thyroid hormone concentrations were observed. LPO was significantly high in 400 mg kg^{-1} body wt ($P < 0.05$) and in 500 mg kg^{-1} body wt ($P < 0.01$) treated animals compared to the control value. CAT activity was also significantly decreased ($P < 0.001$) by these doses. In these groups, there was also a significant decline in both T_3 and T_4 concentrations ($P < 0.001$ for both the hormones). However, in 200 mg kg^{-1} treated group, although T_3 concentration was significantly more, T_4 concentration was reduced ($P < 0.001$ for both).

The results clearly reveal a dose-dependent alteration in thyroid hormone concentrations following the treatment of M. charantia fruit extract. An increase in T_3 concentration by 200 mg kg^{-1} body wt of the plant extract and a decrease by the two higher doses (400 and 500 mg kg^{-1} body wt) suggest that low dose may stimulate the synthesis and/or release of T_3 whereas, higher doses are inhibitory to both the hormones. Although some other plant extracts have already been reported to inhibit thyroid function[4,6], the dose-dependent alterations in thyroid hormone concentrations and in LPO as observed presently following M. charantia extract treatment, is an interesting observation.

Out of the two thyroid hormones, T_4 is synthesized by the thyroid gland, whereas T_3, which is metabolically more potent than T_4, is largely produced by peripheral conversion of the latter hormone in the liver and kidney[14,15]. Therefore, M. charantia-induced alterations in both the thyroid hormones indicate that the plant extract might be acting both at the level of the thyroid gland and at the level of extra-thyroidal tissues, including liver. Interestingly, when hepatic LPO was studied, it was enhanced by the two higher doses of the plant extracts, indicating the hepatotoxic effects of these doses. Changes in the antioxidant enzyme, CAT, also corroborate with that of LPO. Since LPO is a deleterious process, increase in LPO and a concomitant decrease in CAT activity in higher dosed groups, reveal that excess use of the extract could be harmful.

Although other mechanisms of action(s) of M. charantia cannot be ruled out, from the present findings it is evident that chronic use of moderate amounts of alcoholic extract of M. charantia fruit may not cause hepatic damage, but the higher doses may prove to be deleterious, at least with respect to thyroid function and hepatic LPO.
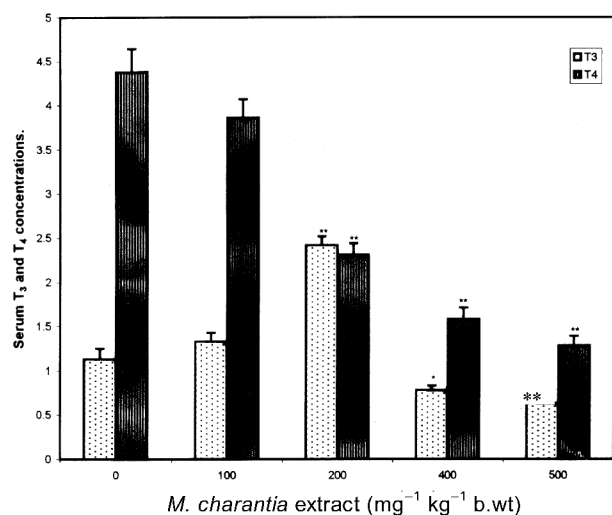

Figure 1. Effect of M. charantia fruit extract (100, 200, 400 and 500 mg kg^{-1} body wt for 15 days) on serum concentrations of T_3 (ng ml^{-1}) and T_4 (ng ml^{-1} × 10) in male mice. Vertical lines indicate SEM. $*P < 0.05$ and $**P < 0.001$ compared to the respective control values.

Table 1. Effect of four different doses of alcoholic extract of M. charantia fruits (100, 200, 400 and 500 mg kg^{-1} body wt) daily for 15 days in the alterations in hepatic LPO (nmol of MDA formed h^{-1} mg^{-1} protein), SOD (units mg^{-1} protein) and CAT (μmol of H_2O_2 decomposed per min per mg protein) activities in male mice. Data have been expressed as mean ± SEM, $n = 8$ in each group

Group	LPO	SOD	CAT
Control	0.623 ± 0.042	5.250 ± 0.208	47.560 ± 1.820
Alcoholic extract treated			
100 mg kg^{-1} b.wt	0.733 ± 0.092	5.501 ± 0.138	48.101 ± 0.940
200 mg kg^{-1} b.wt	0.566 ± 0.071	6.031 ± 0.224	50.931 ± 1.080
400 mg kg^{-1} b.wt	0.914* ± 0.099	4.880 ± 0.529	24.760*** ± 0.941
500 mg kg^{-1} b.wt	1.260** ± 0.166	4.231 ± 0.279	27.840*** ± 0.540

$*P < 0.02$; $**P < 0.01$; $***P < 0.001$ compared to the respective control values.

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Role of nitrification inhibitors on nitrous oxide emissions in a fertilized alluvial clay loam under different moisture regimes

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Fertilized soil is considered to be a major source of nitrous oxide (N_2O) in the atmosphere. In a laboratory incubation experiment, emission of N_2O was studied from a clay loam soil fertilized with urea alone and urea combined with nitrification inhibitors, viz. dicyandiamide (DCD) and thiosulphate, at different moisture regimes. Emission of N_2O was observed from day 1 and was appreciable during the first 2 weeks and decreased subsequently. Soil at 80% max. water holding capacity (WHC) had highest total N_2O-N emission followed by soil at field capacity and submergence. Total emissions from control (no N), urea, urea combined with DCD (urea–DCD) and thiosulphate (urea–thiosulphate) were 78.88, 744.39, 415.64 and 654.75 μg N_2O-N kg^{-1} soil, respectively at 80% max. WHC, while total emissions from the corresponding treatments under submergence and at field capacity were 31.7, 298.5, 138.4, 272.8 and 54.6, 333.7, 217.8, 313.5 μg N_2O-N kg^{-1} soil, respectively. Of the applied N, nitrogen lost through total N_2O emissions was 1.06, 0.54 and 0.92% at 80% max. WHC; 0.45, 0.26 and 0.41% at field capacity and 0.43, 0.17 and 0.39% under submergence from urea, urea–DCD and urea–thiosulphate, respectively. Thus, addition of DCD reduced total N_2O-N emission to the extent of 60, 41.5 and 49.4% under submergence, at field capacity and at 80% max. WHC, respectively, when compared to urea alone, while the corresponding reductions on addition of thiosulphate were 9.6, 7.2 and 13.5%.

NITROUS oxide (N_2O) in the atmosphere is a trace gas responsible for global warming and ozone depletion in the stratosphere[1,2]. Atmospheric N_2O concentration has increased from 280 to 290 ppbv (ref. 3) during the pre-industrial period to 311 ppbv in 1996 (refs 4 and 5) and is responsible for 5% of total greenhouse effect[6]. Soil is considered as one of the major sources of N_2O along with industrial emissions. Agricultural soils contribute 20 to 70% of anthropogenic N_2O emissions[7]. Soil supplied with both industrially and biologically fixed nitrogen emits N_2O during denitrification[8] and nitrification[9]. Moisture regime of the soil is an important factor influencing N_2O emission by regulating oxidation and reduction reactions. The primary effect of water on denitrification in aerobic and anaerobic soil is to restrict O_2 levels by reducing the air–water interfacial area within pore space[10] and hence the supply of oxygen[11]. Excess water not only limits O_2 diffusion into the soil, but also affects the movement, distribution and relative proportion of evolved denitrification gases[12]. Maximum N_2O flux was recorded in soil moisture content between 18 and 22% (ref. 13).

Production of N_2O from denitrification and nitrification is more in N-fertilized systems[14]. Use of nitrification inhibitors is regarded as one of the options to reduce N_2O emission from agriculture. They can reduce emission of N_2O directly by reducing the rate of NH_4^+ oxidation to NO_3^- and therefore reducing the N_2O loss associated with nitrification, or indirectly by reducing the amount of NO_3^- available for denitrification[15]. In this communication, the role of different nitrification inhibitors (DCD and thiosulphate) on N_2O emission at different moisture regimes is discussed.

Surface soil sample (0–15 cm) was collected from the research farm at the Indian Agricultural Research Institute (IARI), New Delhi. The soil sample was air dried, crushed and passed through a 2 mm sieve before incubation. The soil properties are presented in Table 1. The soil (40 g) was incubated in 200 ml airtight spoutless beakers (in triplicate) in a BOD incubator at 30 ± 1°C for 37 days. To monitor N_2O emission, nitrogen was applied @ 62.5 mg N kg^{-1} soil in the form of urea, urea combined with DCD (urea–DCD) and urea combined with thiosulphate (urea–thiosulphate). No nitrogen was