

IP6 and Human Breast Cancer

Vucenik et al. demonstrated experimental evidence that IP6 (inositol hexaphosphate) from rice bran inhibit the growth and stimulates apoptosis of human breast cancer cells in addition to preventing colon cancer. Stabilized rice bran derivatives are rich in IP6. The consumption of stabilized rice bran derivatives may be advantageous with respect to cancer prevention.



Inhibition of rat mammary carcinogenesis by inositol hexaphosphate (phytic acid). A pilot study

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Abstract

Since phytic acid (inositol hexaphosphate, InsP_6) and inositol (Ins) have been demonstrated to have anti-tumor and anti-cell proliferative action in several experimental models of carcinogenesis, in a pilot study we have examined their effect on 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumor model. Starting a week prior to induction with DMBA, the drinking water of female Sprague-Dawley rats was supplemented with either: 15 mM InsP_6 , 15 mM Ins, or 15 mM InsP_6 + 15 mM Ins; a control group received no inositol compounds. Animals (55-day-old) were given a single dose of DMBA (20 mg) in 1 ml of sesame oil by oral intubation. Four additional groups not receiving DMBA, but drinking tap water, InsP_6 , Ins, or InsP_6 + Ins of the same molarity as experimental groups were observed for the duration of the study to monitor for any putative toxicity following this long-term treatment. As opposed to the DMBA-only group, rats treated with InsP_6 \pm Ins showed a 48% reduction in the number of tumors/tumor bearing animal (tumor multiplicity) and a 40% reduction in the number of tumors/rat. In contrast to 20% rats in DMBA-only group, only 0-8% animals in the treatment group had 5 or more tumors. Likewise, the tumor incidence was reduced by 19% in InsP_6 \pm Ins as compared to control untreated animals. The tumors in the treated groups were also 16% smaller in size. Data from this pilot study suggest that in addition to being effective against colon cancer, InsP_6 \pm Ins may be protective against mammary carcinoma as well; additional studies are however warranted.

Key words: Carcinogenesis; Inositol hexaphosphate; Sprague-Dawley rats; Pilot study

1. Introduction

It is now a well recognized fact that diet plays a role in the etiology of certain cancers, particularly cancers of the colon, breast, prostate and pan-

creas. Inositol hexaphosphate (InsP_6 or phytic acid), is a component of a cereal diet which may be one of the active ingredients responsible for the protective effect of these 'high fiber' diets. Recent studies by Shamsuddin and co-workers have shown that InsP_6 inhibits experimentally-induced colon carcinogenesis in both rats and mice [1-5]. Concomitant to this tumor inhibition there is decreased cell proliferation. These results were

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Abbreviations: DMBA, 7,12-dimethylbenz(a)anthracene; Ins, myo-inositol; InsP_{1-6} , inositol monophosphate through hexaphosphate; InsP'_s , inositol phosphates.

confirmed by Pretlow et al. [6] in a rat model of colon cancer. Interestingly, inositol (Ins), the parent carbohydrate of InsP_6 and also naturally occurring, potentiates the anti-cancer action of InsP_6 [3,4]. That the antineoplastic action of $\text{InsP}_6 \pm \text{Ins}$ is not restricted to colon has been demonstrated by us and others. For example, Jariwalla et al. [7] in a rat fibrosarcoma tumor model, and Vucenik et al. [8] in a model of mouse transplantable tumor have shown that InsP_6 has a tumor inhibitory activity; Hirose et al. [9] report of hepatocellular carcinoma inhibition with InsP_6 . Thompson and co-workers [10,11] confirmed that InsP_6 decreases colonic epithelial cell proliferation and have also shown a reduction of early markers of experimental mammary carcinogenesis. In the *in vitro* models InsP_6 inhibited tumor cell growth and increased cell differentiation and maturation of HT-29 human colon carcinoma [12] and K562 human erythroleukemia cell lines [13].

An extension of the study to other organ systems is timely and worthwhile. Breast carcinoma is one of the most frequent causes of death among women [14], the most common cancer in North American women and the second leading cause of cancer death (surpassed only by lung cancer), affecting men as well. Epidemiological studies have shown a close relationship between colon and breast cancers and it is possible that they may have some common etiologic factors.

It has been suggested that high dietary fiber intake can modify the otherwise adverse effect of a diet high in animal fat on breast cancer risk [15–17]. There is support from some, but not all case-control studies for the postulated relationship between breast cancer risk and intake of dietary fiber [18]. Graf and Eaton speculated that InsP_6 and not fiber may be responsible for the inverse correlation between the incidence of colon cancer and the consumption of fiber-rich foods [19]. When the InsP_6 content of cereals, fruits, and vegetables is considered, the international data suggest that there is a greater negative correlation between InsP_6 and colon cancer incidence than between fiber and colon cancer incidence [19,20].

Because of the (a) occurrence of Ins and InsP_{1-6} almost in all mammalian cells; (b) involvement of InsPs , particularly InsP_3 and InsP_4 in the regula-

tion of cell function, growth and differentiation, and (c) their antitumor action in various experimental models of cancer, we conducted a pilot study to test whether $\text{InsP}_6 \pm \text{Ins}$ would be effective in an experimental mammary carcinoma model. We report that InsP_6 alone and in combination with Ins reduces 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in female Sprague–Dawley rats and that long-term treatment with InsP_6 has no toxic side effect on the recipients.

2. Materials and methods

2.1. Tumor induction

Twenty milligrams of DMBA in 1 ml of sesame oil (both from Sigma Chemical Co., St Louis, MO) was given to fifty 5-day-old female Sprague–Dawley rats (Charles River, Boston, MA) as a single dose via intragastric intubation. Vehicle controls received an equal volume of sesame oil. All animals were maintained on laboratory chow and were housed in groups of five in suspended metal cages in a temperature- and humidity-controlled facility on a 12-h-light, 12-h-dark cycle. The animals were handled as a biohazard for 7 days after DMBA administration.

2.2. Treatment of experimental animals

The treatment with Ins and InsP_6 was started a week before the carcinogen was given; the treatment was continued for the duration of the experiment (for an additional 16 weeks). Experimental groups were treated with 15 mM InsP_6 ($n = 21$, dodecasodium salt from corn, Sigma Chemical Co., St Louis, MO), 15 mM Ins ($n = 25$, Sigma Chemical Co., St Louis, MO), and 15 mM $\text{InsP}_6 + 15$ mM Ins ($n = 21$) administered in drinking water. The pH of the solutions was adjusted to 7.3–7.5. A control group was given tap water ($n = 10$). The amount of fluid (tap water or water containing $\text{InsP}_6 \pm \text{Ins}$) consumption was carefully monitored. Drink intakes of rats assigned to each treatment were determined during the first 8 weeks of the experiment and were used to estimate the amount of Ins compounds ingested per day. To check the possible side-effects of long-term treatment, additional 4 groups ($n = 5-6$ each) of nega-

tive controls (animals not receiving DMBA) were included in this experiment: one group was given tap water to drink, and others received 15 mM InsP_6 , 15 mM Ins, 15 mM InsP_6 + 15 mM Ins. These animals were kept for the same duration as the experimental DMBA-treated animals, body weight was monitored weekly and the animals were sacrificed at the same time as the experimental groups. The serum level of Ca^{2+} and Mg^{2+} were determined by Ectachem-700 (Kodak Co, Rochester, NY), Fe^{2+} was measured spectrophotometrically, while serum Zn^{2+} content was determined by atomic absorption spectroscopy.

2.3. Tumor measurement

Body weights, tumor incidence, and measurements were recorded weekly throughout the experimental period. The mammary gland regions were palpated to detect the presence of nodules or any other abnormalities. Each tumor location was recorded and the size was measured with a vernier caliper in 2 perpendicular dimensions. Tumor diameter was calculated by averaging these 2 measurements. Weekly tumor measurements were added up for each rat, and the values were expressed by summing the average diameter of all tumors for tumor-bearing rats of each group. Assessments of tumor incidence (percentage of rats with tumor) and multiplicity (number of tumors per animal and number of tumors per tumor-bearing animals) were also done. At autopsy, all of the mammary glands were exposed to detect palpable and non-palpable tumors. All of the visible and palpable lesions were excised, fixed in buffered formalin, blocked in paraffin, sectioned and stained with hematoxylin and eosin, and examined with a light microscope. Please note that the data as presented portray all of the tumors, palpable and impalpable.

2.4. Statistical analysis

Differences in mammary tumor size, average tumor number, mean tumor latent period, mineral content, body weights and water consumption were analyzed by Student's *t*-test. Statistical difference in tumor incidence was analyzed by the χ^2 test. The analysis for significance of difference in tumor number/animal in each group was carried out by Wilcoxon-Whitney rank test. Differences

in the overall frequency of tumors per rat (including tumor-free rats) and in the number of tumors per tumor-bearing rat were also assessed by Armitage's test for trends in proportions [21].

3. Results

3.1. Body weight and histology of tumors

Weekly total body weight measurements of the animals did not show a significant difference between groups of rats either with or without DMBA (Figs. 1 and 3). Animal weight gains were not affected by the treatment, until the last part of the experiment, when a small but statistically non-significant increase in the body weight gain in DMBA groups was observed (Fig. 1). This difference reached a level not greater than 10% and may have been due to the greater number of tumors. The average daily consumption of Ins and InsP_6 by rats on DMBA (Ins = 32.96 ± 2.68 ml, 0.49 mmol; InsP_6 = 28.73 ± 2.55 ml, 0.43 mmol) and control groups (Ins = 42.22 ± 6.27 ml, 0.63 mmol; InsP_6 = 29.81 ± 4.31 ml, 0.45 mmol) were not significantly different. It is interesting that the rats preferred to drink Ins over InsP_6 . Tap water intake was 28.49 ± 3.03 ml. Histopathological evaluation of mammary tumors demonstrated that except for one fibroadenoma and one hyperplasia, the rest of the 144 tumors were adenocarcinomas.

3.2. Tumor incidence, multiplicity, and size

Compared to the untreated rats, the rats treated with inositol compounds show a 19% reduction in tumor incidence, a 39% reduction in number of tumors per group, 40% per rat, and 48% per tumor bearing rat at the end of 16 weeks after DMBA instillation (Table 1). However, these reductions though marked were not statistically significant ($0.05 < P < 0.10$). While the tumor multiplicity (i.e., the number of tumors per tumor bearing rats) was reduced by half, the tumor diameter was reduced by 16% only; very similar was the reduction in the tumor volume (data not presented). The majority of animals in the treatment groups (Ins P_6 \pm Ins) had 1–2 tumors (Table 2). When the tumor burden (multiplicity) was analyzed, it was found that 20% of rats on DMBA alone had ≥ 5 tumors; in marked contrast none of the

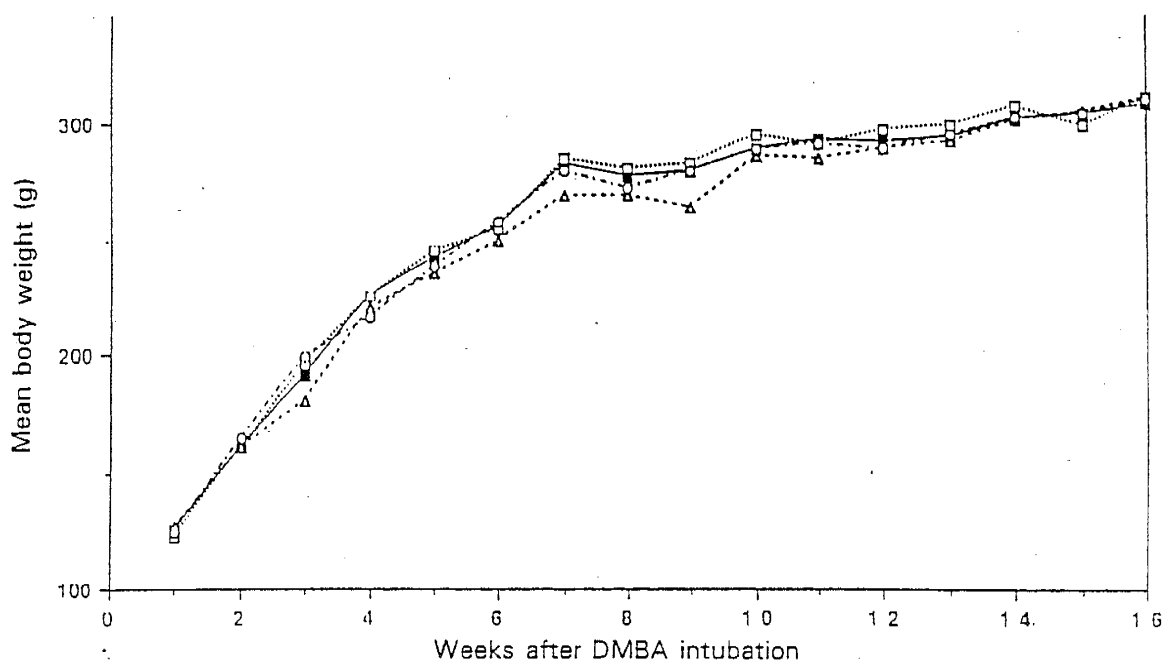


Fig. 1. Effects of inositol compounds treatment on the body weight of rats treated with InsP₆ (□) (*n* = 21), Ins (Δ) (*n* = 25), and InsP₆ + Ins (○) (*n* = 21) after DMBA intubation. DMBA-only group (■) (*n* = 10).

animals treated with InsP₆ ± Ins, and only 4.8% InsP₆ treated animals had ≥ 5 tumors. The effect of treatment on DMBA-induced mammary carcinogenesis is further illustrated in Fig. 2 showing the cumulative weekly incidence of tumors in each group of rats after administration of DMBA. The time course of palpable tumor development in all

treated groups exhibited a marked reduction in tumor yields in comparison with the group given DMBA-only, but statistically non-significant ($0.05 < P < 0.10$).

3.3. Toxicity

Fig. 3 shows that long-term administration of 15

Table 1
Effect of inositol compounds on tumorigenesis of DMBA-induced rat mammary carcinoma

Treatment	Number of rats	Number of rats with tumors	Number of tumors	Number of tumors per rat	Number of tumors per tumor-bearing rat	Tumor diameter (mm)	Rats with ≥ 5 tumors (%)
DMBA	10	7	28	2.8 ± 1.2 ^a	4.0 ± 1.6 ^a	12.6 ± 1.7	20.0
DMBA + 15 mM InsP ₆	21	17	36	1.7 ± 0.3	2.1 ± 0.3	12.4 ± 1.7	4.8
DMBA + 15 mM Ins	25	20	43	1.7 ± 0.3	2.2 ± 0.3	10.6 ± 1.2	8.0
DMBA + 15 mM InsP ₆ + 15 mM Ins	21	12	37	1.8 ± 0.4	3.1 ± 0.3	10.8 ± 1.3	0.0

^aDenotes mean ± standard error.

Table 2
Effect of inositol compounds on DMBA-induced rat mammary carcinoma

Treatment	Number of tumors per group			
	0	1-2	3-4	≥5
DMBA	30.0% (3) ^a	30.0% (3) ^a	20.0% (2)	20.0% (2)
DMBA + InsP ₆	19.0% (4)	52.4% (11)	23.8% (5)	4.8% (1)
DMBA + Ins	20.0% (5)	56.0% (14)	16.0% (4)	8.0% (2)
DMBA + InsP ₆ + Ins	42.9% (9)	19.0% (4)	38.1% (8)	0% (0)

^aDenotes the number of animals.

mM InsP₆ (pH 7.4), 15 mM Ins (pH 7.4), or 15 mM InsP₆ + 15 mM Ins (pH 7.4) did not have significant effects on the body weight of experimental animals. As shown on Table 3, this treatment had no effect on the levels of various serum minerals (Ca²⁺, Fe²⁺, Mg²⁺, Zn²⁺).

4. Discussion

The results of this study demonstrate a notable, albeit modest reduction in the frequency, size and

incidence of mammary carcinoma as a result of InsP₆ ± Ins treatment. Since in this study a very high dose of DMBA (20 mg) was used, and the number of animals was small, not surprisingly the results were not statistically significant. The wide variation in tumor sizes in animals exposed to DMBA may be due to the fact that in our calculations both palpable and non-palpable tumors were included (other studies were based on the palpable tumors only, excluding the non-palpable tumors from data analysis).

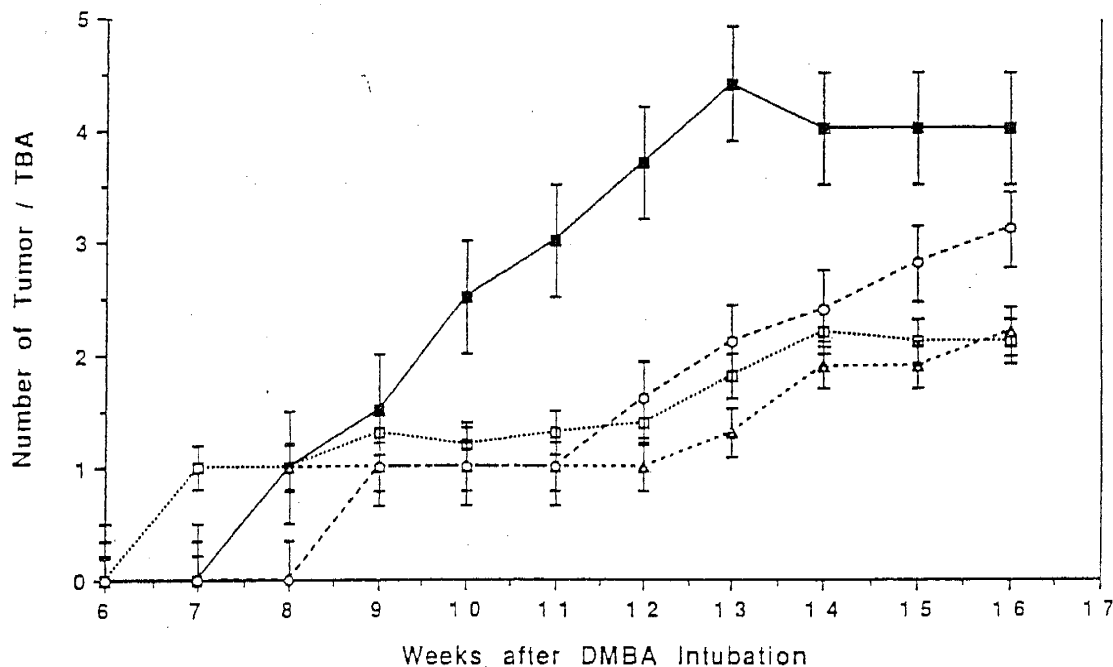


Fig. 2. Effect of dietary inositol compounds on the initiation of rat mammary tumors by DMBA. DMBA, (■); DMBA + InsP₆, (□); DMBA + Ins, (Δ) and DMBA + InsP₆ + Ins, (○). The rats were started on this treatment one week before intragastric instillation of DMBA and maintained on the diet throughout the length of the experiment. The significance level at 10-16 weeks post DMBA-treatment is $0.05 < P < 0.10$. S.D. is $\leq 10\%$ of mean.

as Fe^{2+} and Ca^{2+} [28]; Thompson and Zhang [11] thus hypothesized that its effect may be partly related to its mineral binding ability. Last but not least is the immunological route, $\text{InsP}_6 \pm \text{Ins}$ has been found to augment at natural killer (NK) activity in vitro and normalize the carcinogen-induced depression of NK activity in vivo [4].

Very few laboratory animal studies dealing with the relationship between dietary fiber intake and the development of mammary cancer are reported [for review see ref. 16]. Our study was carried out using isolated compounds that are associated with high fiber. Because the components of food interact with each other, the potential benefit of Ins compounds could be extended for cancer prevention. The components of high fiber diet may affect the development of cancers of the gastrointestinal tract and the breast through bile acids [29], caloric restriction [16,29], and through influence on estrogen metabolism, decreasing circulating estrogen, acting as a phytoestrogen, removing fat in the feces with its high binding property [17,29].

Most nutritional interest thus far focused on the inhibitory effect of InsP_6 on mineral absorption (however, the ability to bind metal ions, particularly iron, may provide the basis for the anticarcinogenic effects of this compound). It has been generally argued, that because of the chelating potential of InsP_6 , it forms complexes with polyvalent cations, reducing solubility of nutritionally important minerals, therefore affecting their bioavailability. In an additional four groups of rats, we monitored the effect of $\text{InsP}_6 \pm \text{Ins}$ on body weight gains, food intake, general appearance of animals, and serum level of various divalent cations and our results show that these compounds are virtually innocuous.

The inhibitory effect of $\text{InsP}_6 \pm \text{Ins}$ on DMBA-induced mammary carcinoma albeit modest, is encouraging. Since naturally occurring carcinogens are relatively weak by virtue of being at a low concentration as compared to experimental models, the anti-tumor effect of $\text{InsP}_6 \pm \text{Ins}$ may be more marked and statistically significant if a lower dose of DMBA is used. Further work and additional investigations with better study design are needed to confirm our preliminary observations, and fully understand the mechanism(s) of this action.

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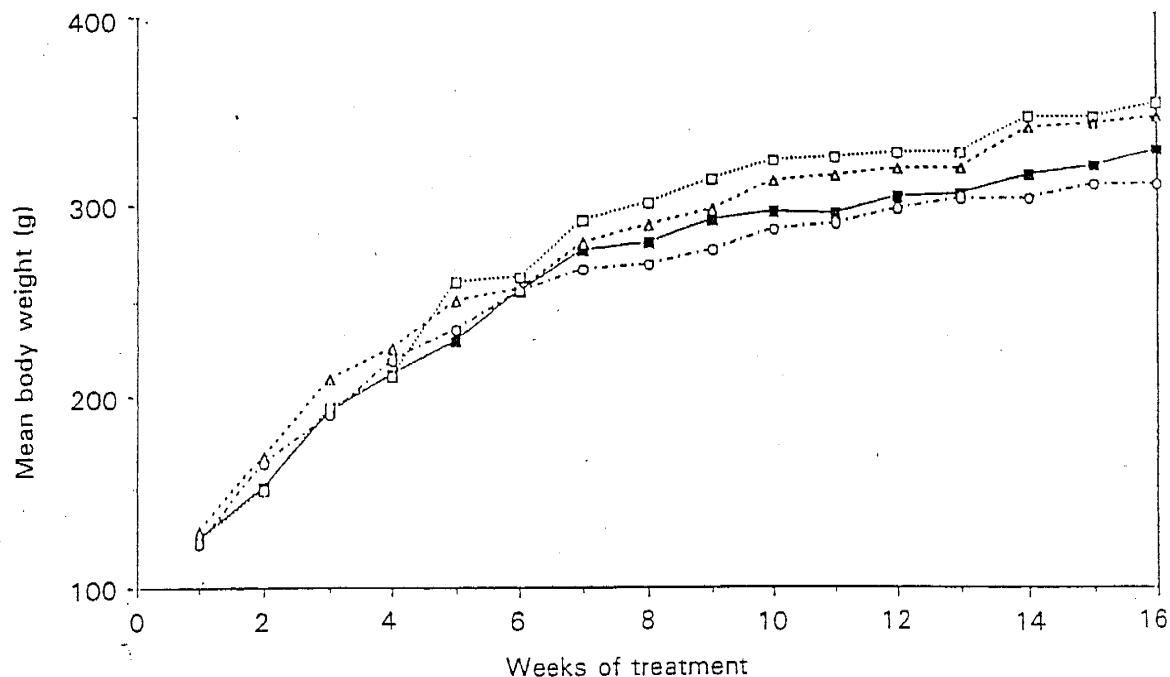


Fig. 3. Cumulative weight gain curves of the mean value for each control group plotted versus time. The differences between various groups were not significant at any time point: when compared to DMBA-treated groups (Fig. 1) there was not significant difference either. Tap water. (■); InsP₆. (□); Ins. (Δ); InsP₆ + Ins. (○). S.D. is $\leq 10\%$ of mean.

The exact mechanism(s) through which InsP₆ \neq Ins exert their anticancer action are not known and remains to be determined. We hypothesize that one of the several ways by which InsP₆ \neq Ins exert their action is through lower InsP levels. Measurement of intracellular InsP levels following InsP₆ treatment of malignant cells do indeed show an increased level of lower InsP values [13,22]. Of note is that virtually all animal cells contain InsP₁₋₆ and lower InsP levels, especially InsP₃ have important roles in cellular signal transduc-

tion, regulation of cell function, growth and differentiation [23-26]. The observed anticancer effect of Ins compounds could be mediated through several other mechanisms. Since DMBA-induced mammary cancer is thought to operate through the formation of free radicals, InsP₆ could interfere with free radical generation [27] and therefore inhibit mammary carcinogenesis, but that alone does not explain why Ins would be effective. By virtue of the highly negatively charged nature of InsP₆ it can bind divalent cationic minerals such

Table 3
Effect of inositol compounds on serum minerals

Treatment	Ca ²⁺ (mg/dl)	Fe ²⁺ (mg/dl)	Mg ²⁺ (mg/dl)	Zn ²⁺ (mg/dl)
Tap water (n = 6)	10.2 \pm 0.7 ^a	305.0 \pm 61.9 ^a	3.1 \pm 0.6 ^a	82.3 \pm 20.0 ^a
15 mM InsP ₆ (n = 3)	11.1 \pm 1.1	376.0 \pm 35.4	4.2 \pm 1.2	107.0 \pm 38.2
15 mM Ins (n = 4)	9.7 \pm 0.5	430.5 \pm 9.2	3.0 \pm 0.9	74.5 \pm 14.8
15 mM InsP ₆ + 15 mM Ins (n = 5)	9.9 \pm 0.2	316.8 \pm 37.4	2.7 \pm 0.4	88.8 \pm 3.5

^aDenotes mean \pm S.D.