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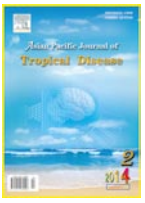

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Toxicological and safety evaluation of *Nigella sativa* lipid and volatile fractions in streptozotocin induced diabetes mellitus

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ABSTRACT

Objective: To evaluate the toxicological aspects of *Nigella sativa* (*N. sativa*) lipid and volatile fractions in streptozotocin induced diabetes mellitus.

Methods: National Institute of Health (NIH), Islamabad provided us thirty Sprague Dawley rats that were further divided into three groups, *i.e.* control, *N. sativa* lipid fraction (4%) and *N. sativa* volatile fraction (0.3%), respectively. The serological and haematological indices were evaluated at 4-week intervals during 56 d study.

Results: The results indicated that the diabetes mellitus imparted negative effects on various serological and haematological attributes. However, supplementation of the *N. sativa* lipid fraction and *N. sativa* volatile fraction ameliorated the adverse consequences of diabetes mellitus. The diabetes induced renal toxicity and imbalanced serum chemistry were slightly modulated by experimental diets. However, the impact of essential oil was more significant as compared to the fixed oil.

Conclusions: In a nutshell, experimental diets containing *N. sativa* lipid fraction and *N. sativa* volatile fraction are effective without having any toxicological effects, and experimental diets reduced toxicological and adverse consequences of diabetes mellitus.

1. Introduction

The medicinal plants are in use since ancient times for prevention and cure of various maladies. Considering the information disseminated from ancestors, researchers over the globe carried out studies to explore the role of various plant against diabetes mellitus[1,2]. The World Health Organization (WHO) published a report in 2004 that the number of diabetes patients will reach to 350 million at the end of 2025. According to an estimate, 30%-40% of lifestyle related disorders are avoidable by advising masses to adopt healthy lifestyle with proper diet diversifications strategies[3]. The industrialization in the last century resulted in widespread dependence of humans on mechanical tools, which led to limited physical activity and poor dietary habits. These often lead to progression and pathogenesis of lifestyle related disorders. The medicinal plants uniquely combine the concepts of medication and diet together. However, controlling diabetes mellitus and other lifestyle related disorders requires multidimensional approaches[4-6].

Diet selection and inclusion of selective medicinal plant is imperative for the management of diabetes and its allied complications. Some anti-diabetic compounds are also present in some plants especially from peel of potato, *Bougainvillea*, *etc*. The food containing high fibre contents like oat, guar gum and psyllium husk *etc*. are effective in weight management thus reducing the extent of diabetes mellitus[7]. Antioxidants, phytosterols and flavonoids have shown hypoglycemic and hypocholesterolemic potential[8]. Black cumin [*Nigella sativa* L. (*N. sativa*)] contains various bioactive molecules that might be helpful in reducing the

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risk of various ailments. In some countries, it is widely used as herbal medicine by various cultures and civilizations to treat and prevent number of ailments[9,10]. *N. sativa* lipid and volatile fractions holds insulinotropic properties and maintains β-cells integrity that enhances the insulin production important in mediating diabetes mellitus[11].

The nutritional strategies can improve the quality of life in diabetic patients.

Safety evaluation through animal modeling studies along with establishing their toxicity limits are essential to determine the healthiness of novel food products. Recent global scenario also encompasses the findings of clinical pathological trials in test animal towards the safe status with specific health claim[12]. In this article, the authors studied the effects of streptozotocin on various serological and haematological indices. The effects of *Nigella sativa* (*N. sativa*) lipid and valatile fractions were assessed over a period of 56 d. The findings will be beneficial in validating the traditional use of *N. sativa* to treat diabetes mellitus along with providing evidences to the clinicians and nutritionists regarding its therapeutic potential.

2. Materials and methods

2.1. Plants and reagents

The Barani Agricultural Research Institute, Chakwal provided us with *N. sativa* seeds. Chemical Reagents (analytical & HPLC grade) and standards were purchased from Sigma-Aldrich Tokyo, Japan and Merck KGaA, Darmstadt, Germany.

Following the standard procedures, the seeds of *N. sativa* were slurred with hexane to extract fixed oil, whilst *N. sativa* essential oil was extracted using locally assembled hydro-distillation apparatus. Earlier to this manuscript, the authors published the results pertaining to the nutritional composition of *N. sativa* fixed oil and essential oil in Pakistan Journal of Botany[13], and safety assessment in Food and Chemical Toxicology[14]. The researchers conducted some preliminary trials that helped in finalizing the dosage of the test substances *i.e.* 4.0% *N. sativa* lipid fraction and 0.3% *N. sativa* volatile fraction. The rodents experimental diets containing both fixed and essential oils were prepared and fed for a period of 56 d.

2.2. Animals

National Institute of Health, Islamabad, Pakistan provided infectious free Sprague Dawley rats for the research purpose as per instructions of "Animal Care Committee, National Institute of Food Science & Technology-Faisalabad Pakistan".

The thirty test animals were male rats of 6-7 week old, weighing (130±10) g. They were declared infections and diseases free after physical and behavioral examination. During the one week acclimatization period, basal-diet (AIN-76A) was used to feed rodents and animals were again tested for the same physical and serological examination after acclimatization period. Later, rodents were divided into three groups (10 rats each) and experimental diets were fed for a period of 8 weeks. The animals were maintained according to standard guidelines of Animal Institute of Nutrition, USA, *i.e.* temperature (23±2) °C, relative humidity (55±5)%, and 12-h light-dark cycle. In the first week, the feed of the rats was basal diet in order to acclimatize them to new environment. Later, rats received their respective experimental diets for a period of eight weeks (56 days).

Drinking water was provided *ad libitum* in polypropylene bottles with stainless steel sipper tubes. At 28 and 56 d of feeding trials, five rats from each group were decapitated for blood collection through cardiac and neck puncture[15]. Organs like left and right kidney, pancreas, spleen, heart, lungs, liver, and pancreas were separated from the body using the standard procedures and organ to body weight ratios were calculated after measuring their weights. The collected blood samples were analyzed for further assays and details are mentioned herein.

2.3. Serum proteins and electrolytes profile

Conjugated and un-conjugated bilirubin proteins were determined by Jendrassik-Grof method[16]. Moreover, serum total proteins, albumins, globulin and albumin-globulin (A/G) ratio were determined using their respective kits obtained from Sigma-Aldrich Chemicals Co. The levels of electrolytes were also assessed to check the safety of the *N. sativa* lipid and volatile fractions.

2.4. Kidney functionality test

The serum samples were analyzed for urea by glutamic dehydrogenase-method and creatinine by Jaffe-method using commercial kits[17], to assess the proper renal functionality and impact of the *N. sativa* lipid and volatile fractions.

2.5. Haematological attributes

Blood samples collected in ethylene diamine tetraacetic acid coated tubes were analyzed for complete blood profile like total red blood cells count, hemoglobin, and hematocrit. Platelets count and erythrocytes sedimentation rates (ESR) were estimated[18]. Total white blood cells, neutrophiles, lymphocytes, monocytes, eosinophiles and basophiles were also determined[19].

2.6. Statistical analysis

Data obtained was analyzed statistically using statistical package *i.e.* Cohort V-6.1. Sample for each analysis was run quadruplet and values were expressed as means±SD. However, means for diets and days were expressed as means ±SEM. The distribution of the data was checked and analysis of variance was applied afterwards to determine the level of significance. The significant differences were further compared through Duncan's multiple range test.

3. Results

The results regarding chemical composition of *N. sativa*

seeds have already been published in Pakistan Journal of Botany[13]. In the same year, the authors published results regarding the safety assessment of *N. sativa* fixed and essential oil in Food and Chemical Toxicology[14]. In the present study, body weight of rats varied significantly as a function of diets and study intervals[9]. The results regarding organ-body weight ratio indicate that the liver to body weight ratio varied from (3.86±0.15) to (4.66±0.27) g/100 g and diets differed significantly (P<0.05) in terms of heart, kidney, pancreas, and spleen weight to body weight ratio (Table 1).

Table 1

Organs to body weight ratio. g/100 g.

Organ	Diets	Study intervals (d)			Means
		0	28	56	
Heart	D1	0.45±0.03	0.48±0.02	0.51±0.03	0.48±0.02[a]
	D2	0.43±0.02	0.40±0.01	0.41±0.02	0.41±0.01[b]
	D3	0.45±0.02	0.42±0.02	0.40±0.02	0.42±0.01[b]
Liver	D1	4.66±0.27	4.44±0.14	4.13±0.21	4.41±0.15
	D2	4.40±0.18	4.44±0.15	4.26±0.19	4.37±0.05
	D3	3.86±0.15	4.34±0.21	4.46±0.20	4.22±0.18
Left Kidney	D1	0.52±0.03	0.51±0.02	0.52±0.03	0.52±0.01[a]
	D2	0.50±0.02	0.49±0.02	0.45±0.02	0.48±0.02[b]
	D3	0.50±0.02	0.47±0.02	0.44±0.02	0.47±0.02[b]
Right Kidney	D1	0.47±0.03	0.50±0.02	0.52±0.03	0.50±0.01[a]
	D2	0.46±0.02	0.42±0.01	0.40±0.02	0.43±0.02[b]
	D3	0.46±0.02	0.45±0.02	0.43±0.02	0.45±0.01[b]
Spleen	D1	0.33±0.02	0.36±0.01	0.35±0.02	0.35±0.01[a]
	D2	0.35±0.01	0.34±0.01	0.30±0.01	0.33±0.02[ab]
	D3	0.32±0.01	0.30±0.01	0.28±0.01	0.30±0.01[b]
Lungs	D1	1.14±0.07	1.08±0.03	1.12±0.06	1.11±0.02
	D2	1.07±0.04	0.99±0.03	1.10±0.05	1.05±0.03
	D3	1.08±0.04	1.03±0.05	1.14±0.05	1.08±0.03
Pancreas	D1	0.68±0.06	0.72±0.04	0.75±0.06	0.72±0.02[a]
	D2	0.65±0.04	0.62±0.04	0.60±0.04	0.62±0.01[b]
	D3	0.64±0.05	0.61±0.05	0.58±0.06	0.61±0.02[b]

D1: Control diet; D2: *N. sativa* lipid fraction; D3: *N. sativa* volatile fraction. Means sharing the same letters in a column do not differ significantly at P<0.05.

The results regarding serum protein profile are presented in Table 2. The diets and study duration affected conjugated and un-conjugated bilirubin significantly (P<0.05). Mean values were in the range of (0.200±0.012) to (0.360±0.030) mg/dL for conjugated bilirubin and (0.560±0.043) to (0.860±0.073) mg/dL for un-conjugated bilirubin (Table 2). Moreover, mean values for urea, creatinine, and uric acid remained in the range of (16.470±1.393) to (20.880±1.484) mg/dL, (0.560±0.047) to (0.900±0.067) mg/dL and (5.190±0.530) to (6.690±0.560) mg/dL, respectively. Creatinine contents varied from (0.560±0.047) to (0.900±0.067) mg/dL during the end of entire study duration. The urea contents increased in control groups showing higher nitrogen metabolisms, whilst experimental groups showed non-significant variations in urea, creatinine, and uric acid contents.

In the present study, total protein contents varied from (6.570±0.250) to (7.740±0.392) mg/dL (Table 2). Level of total serum protein increased non-significantly with the passage of time. Albumin contents increased as a function of study duration (P>0.05), *i.e.* (3.120±0.119) mg/dL at 0 d to (3.850 ±0.171) mg/dL at the end of study (56 d). The maximum globulin contents of (3.410±0.130) mg/dL were recorded in control and the lowest value of (3.040±0.220) mg/dL was recorded in rats fed on 0.3% *N. sativa* volatile fraction. Diets produced momentous difference in A/G ratio in serum of diabetic rats and maximum A/G ratio (1.150±0.062) was recorded in *N. sativa* volatile fraction fed rats, whilst the least A/G ratio of (1.04±0.008) was recorded in control group.

The results regarding the serum electrolytes including sodium, potassium, chloride and bicarbonates, and remained in the range of (113.23±6.53) to (137.68±5.24) mEq/L, (3.80±0.22) to (4.64±0.20) mEq/L, (118.75±5.78) to (138.72±6.16) mEq/L and (25.90±0.99) to (28.97±1.67) mEq/L, respectively (Table 3).

Table 2

Effects of fixed and essential oils of black cumin on liver and kidney function in diabetic rats.

Parameters	Diets	Study intervals (d)			Means
		0	28	56	
Bilirubin conjugated (mg/dL)	D1	0.240±0.020	0.300±0.016	0.360±0.030	0.300±0.035
	D2	0.200±0.012	0.240±0.017	0.270±0.020	0.240±0.020
	D3	0.200±0.015	0.220±0.017	0.240±0.025	0.220±0.012
Bilirubin un-conjugated (mg/dL)	D1	0.680±0.057	0.830±0.045	0.860±0.073	0.790±0.056
	D2	0.590±0.035	0.630±0.045	0.660±0.049	0.630±0.020
	D3	0.620±0.048	0.560±0.043	0.630±0.064	0.600±0.022
Urea (mg/dL)	D1	19.580±1.639	16.540±0.901	16.470±1.393	17.530±1.025
	D2	19.610±1.155	20.880±1.484	17.340±1.295	19.280±1.035
	D3	20.470±1.575	16.850±1.301	19.440±1.986	18.920±1.077
Creatinine (mg/dL)	D1	0.560±0.047	0.820±0.045	0.790±0.067	0.720±0.082
	D2	0.650±0.038	0.720±0.051	0.900±0.067	0.760±0.074
	D3	0.660±0.051	0.750±0.058	0.820±0.084	0.740±0.046
Uric acid (mg/dL)	D1	6.690±0.560	6.200±0.338	5.800±0.490	6.230±0.257
	D2	6.400±0.377	5.700±0.405	5.270±0.394	5.790±0.329
	D3	5.680±0.437	6.200±0.479	5.190±0.530	5.690±0.292
Total proteins (mg/dL)	D1	7.020±0.405	7.530±0.244	7.740±0.392	7.430±0.214
	D2	7.020±0.294	7.210±0.242	7.300±0.322	7.180±0.083
	D3	6.570±0.250	6.950±0.338	7.320±0.325	6.950±0.217
Albumins (mg/dL)	D1	3.320±0.192	3.600±0.117	3.680±0.187	3.530±0.109
	D2	3.300±0.138	3.580±0.120	3.680±0.162	3.520±0.114
	D3	3.120±0.119	3.500±0.170	3.850±0.171	3.490±0.211
Globulins (mg/dL)	D1	3.160±0.182	3.460±0.112	3.600±0.183	3.410±0.130
	D2	3.260±0.136	3.180±0.107	3.180±0.140	3.210±0.027
	D3	3.000±0.114	3.060±0.149	3.070±0.136	3.040±0.022
A/G ratio	D1	1.050±0.061	1.040±0.034	1.020±0.052	1.040±0.008
	D2	1.010±0.042	1.130±0.038	1.160±0.051	1.100±0.044
	D3	1.040±0.040	1.140±0.056	1.250±0.056	1.150±0.062

D1: Control diet; D2: *N. sativa* lipid fraction; D3: *N. sativa* volatile fraction.

Table 3

Serum electrolytes. mEq/L.

Parameters	Diets	Study intervals (d)			Means
		0	28	56	
Sodium	D1	113.23±6.53	136.60±4.43	133.29±6.76	127.71±7.30
	D2	134.78±5.64	137.32±4.62	129.65±5.71	133.92±2.26
	D3	137.68±5.24	129.11±6.29	131.38±5.83	132.72±2.56
Potassium	D1	3.80±0.22	4.45±0.14	4.36±0.22	4.20±0.20
	D2	4.60±0.19	4.16±0.14	4.64±0.20	4.47±0.15
	D3	4.23±0.16	4.13±0.20	4.39±0.19	4.25±0.08
Chlorides	D1	130.13±7.51	125.23±4.06	116.07±5.88	123.81±4.12
	D2	120.08±5.02	123.30±4.14	134.46±5.92	125.95±4.36
	D3	126.71±4.82	118.75±5.78	138.72±6.16	128.06±5.80
Bicarbonates	D1	28.97±1.67	28.75±0.93	26.58±1.35	28.10±0.76
	D2	26.00±1.09	26.28±0.88	26.95±1.19	26.41±0.28
	D3	25.90±0.99	28.20±1.37	28.60±1.27	27.57±0.84

D1: Control diet; D2: *N. sativa* lipid fraction; D3: *N. sativa* volatile fraction.

The results regarding haematology indicated that the red blood cell varied from (6.12±0.31) to (7.74±0.34) 10^6/dL (Table 4). The diabetes mellitus resulted in decreased number of red blood cells and experimental diets showed promise of recovery. Similarly, the maximum hematocrit percentage (45.40±2.05)% was witnessed in *N. sativa* volatile fraction group as compared to the minimum in control (41.12±0.58)%.

Likewise, haemoglobin contents varied from (13.22±0.33) to (14.80±0.60) mg/dL. Maximum platelet count of (5.73±0.37) 10^3/μL was recorded in control group as compared to the lowest of (4.95±0.08) 10^3/μL in *N. sativa* volatile fraction group. ESR varied momentously and experimental diets exhibited significant differences with mean ESR values of (3.73±0.20) in control, whilst *N. sativa* lipid fraction and *N. sativa* volatile fraction behaved alike with mean ESR rate of (4.73±0.33) and (4.73±0.39) mm/Hr, respectively.

Table 4

Red blood cell indices in diabetic rats.

Parameters	Diets	Study intervals (d)			Means
		0	28	56	
Red blood cell (10^6/dL)	D1	7.39±0.43	6.83±0.22	6.12±0.31	6.78±0.37
	D2	7.55±0.32	7.25±0.24	7.16±0.32	7.32±0.12
	D3	7.25±0.28	7.40±0.36	7.74±0.34	7.46±0.14
Hematocrit (%)	D1	41.90±2.42	40.00±1.30	41.47±2.10	41.12±0.58
	D2	44.90±1.88	42.64±1.43	48.20±2.12	45.25±1.61
	D3	41.30±1.57	47.24±2.30	47.66±2.12	45.40±2.05
Hemoglobin (mg/dL)	D1	15.93±0.92	14.60±0.47	13.86±0.70	14.80±0.60
	D2	13.57±0.57	12.55±0.42	13.54±0.60	13.22±0.33
	D3	14.06±0.54	12.24±0.60	14.22±0.63	13.51±0.63
Platelet count (10^3/μL)	D1	5.01±0.29	5.99±0.19	6.20±0.31	5.73±0.37
	D2	5.22±0.22	5.42±0.18	5.82±0.26	5.49±0.18
	D3	4.80±0.18	4.98±0.24	5.06±0.22	4.95±0.08
ESR (mm/Hr)	D1	3.96±0.23	3.90±0.13	3.34±0.17	3.73±0.20
	D2	4.19±0.18	4.68±0.16	5.32±0.23	4.73±0.33
	D3	4.01±0.15	4.84±0.24	5.34±0.24	4.73±0.39

D1: Control diet; D2: *N. sativa* lipid fraction; D3: *N. sativa* volatile fraction.

The results presented in Table 5 depicts that white blood cell varied from (10.70±0.83) 10^3/μL to (13.12±1.34) 10^3/μL. The mean values for lymphocytes, neutrophiles, and monocytes ranged from (77.60±4.57)% to (81.70±6.91)%, (12.12±1.02)% to (16.46±0.97)% and (2.90±0.22)% to (3.58±0.37)%, respectively. However, eosinophiles (%) differed significantly as a function of diets and *N. sativa* lipid fraction group had maximum eosinophiles (1.46±0.15)% as compared to the least (1.25±0.02)% in *N. sativa* volatile fraction group.

Table 5

White blood cells indices in diabetic rats.

Parameters	Diets	Study intervals (d)			Means
		0	28	56	
WBC (10^3/μL)	D1	11.60±0.97	10.89±0.59	10.94±0.93	11.14±0.23
	D2	10.88±0.64	12.89±0.92	11.28±0.84	11.68±0.61
	D3	11.14±0.86	10.70±0.83	13.12±1.34	11.65±0.74
Lymphocytes (%)	D1	78.79±6.60	80.39±4.38	81.70±6.91	80.29±0.84
	D2	77.60±4.57	79.60±5.66	79.75±5.96	78.98±0.69
	D3	79.95±6.15	79.23±6.12	78.18±7.99	79.12±0.51
Neutrophiles (%)	D1	14.62±1.22	13.97±0.76	12.12±1.02	13.57±0.75
	D2	16.46±0.97	14.03±1.00	13.87±1.04	14.79±0.84
	D3	14.80±1.14	15.26±1.18	15.49±1.58	15.18±0.20
Monocytes (%)	D1	3.15±0.26	2.96±0.16	2.97±0.25	3.03±0.06
	D2	2.96±0.17	3.51±0.25	3.07±0.23	3.18±0.17
	D3	3.03±0.23	2.90±0.22	3.58±0.37	3.17±0.21
Eosinophiles (%)	D1	1.35±0.11	1.30±0.07	1.31±0.11	1.32±0.02
	D2	1.49±0.09	1.19±0.08	1.70±0.13	1.46±0.15
	D3	1.28±0.10	1.21±0.09	1.25±0.13	1.25±0.02
Basophiles (%)	D1	1.000±0.084	0.770±0.042	0.640±0.054	0.803±0.105
	D2	0.820±0.048	0.620±0.044	0.550±0.041	0.663±0.081
	D3	0.310±0.024	0.490±0.038	0.620±0.063	0.473±0.090

D1: Control diet; D2: *N. sativa* lipid fraction; D3: *N. sativa* volatile fraction.

4. Discussion

Diabetes mellitus is one of the most common non-communicable diseases that targets multi-organ systems. In the recent years, many scientific research interventions explored some novel food sources for their hypoglycemic potential[1]. However, safe appraisal is essential through animal and cohort studies so that physicians and medical practitioners could use them as pharmaceutical drugs against diabetes mellitus. Streptozotocin has been widely used for inducing diabetes in a variety of animals[19,20]. In the present study, induction of diabetes mellitus caused reduction in feed intake; reflected by body weight of rats that decreased with the passage of time. Alimohammadi *et al*. also enumerated the clinical significance of *N. sativa* and presented some evidences from histopathology in diabetic rats[21]. The *N. sativa* lipid and volatile fractions showed slight anorexic effects as weight gain was higher in control as compared to the experimental diets. The results were in close proximity with Kanter *et al*. as they also reported the anorexic effects of black seed supplementation in their experiment related to insulin sensitivity[22]. The results regarding organs weight and organ to body weight further enumerated the increase in heart to body weight ratio which is an indicator of cardiac hypertrophy diabetic rats. This might be due to accumulation of cholesterol, triglycerides, phospholipids, and glycated protein in the myocardium[23]. Ye *et al*. cited values for organs to body weight ratio and the results are in close proximity with the present data set[12]. In the similar studies conducted in the rats using black seeds consumption, Al-Wafai *et al*.[24] and Sultan *et al*.[9] also observed the organ to body weight ratios, however, the variations are attributed to the age of the animal and diabetes mellitus[12].

The toxicological studies involving test animal, the parameters linked with the proper functionality of the liver are of considerable importance[25]. The authors published the results regarding liver functioning test and myocardial infraction and values for these traits were higher than normal rats but *N. sativa* mitigated some of the damage caused by diabetes mellitus. Safety studies conducted by Bamosa *et al*.[23]and Ye et al.[12] reported the normal values for parameters observed in the present study. However, the results were different as diabetes mellitus results in various pathological events resulting in renal toxicity, myocardial infraction, immune dysfunction, etc. Nutritional strategies employing plants and their bioactive molecules are effective along with the practical application of optimum nutrition.

The results regarding various serological and haematological indices showed adverse consequences but *N. sativa* fixed and essential oils showed potential to mitigate some of the damage. However, it should be kept in mind that hyperglycemia is closely linked with free radicals production that ultimately results in protein glycation, and oxidative stress thus damaging various organs. Overall, the findings of present study are useful for the researchers and nutritionist to further conduct population based studies using *N. sativa* fixed and essential oil. The results of such studies would farther validate the present findings.

In a nutshell, health claims associated with *N.*

sativa lipid and volatile fractions are of significance importance. The findings revealed less toxicological effects of experimental diets on serological and haematological attributes thus the *N. sativa* lipid fraction and *N. sativa* volatile fraction can be used to supplement the daily diet of diabetic patients for improving their quality of life. However, authors still consider that similar studies should be replicated in cohort studies involving diabetic patients. Such studies would be more useful for medical specialists and nutritionist to validate the present findings.

Conflict of interest statement

We declare that we have no conflict of interest.

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