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Original Report

Safety evaluation studies on Garden cress (*Lepidium sativum* L.) seeds in Wistar rats

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Summary: Garden cress (*Lepidium sativum* L.) leaves and seeds are used in India as food supplement and also in traditional medicine. We have assessed the safety of Garden cress (GC) seeds by conducting acute and subchronic toxicity studies in adult Wistar rats. For the acute toxicity study, 0.5 –5.0 g/kg body weight of the GC seed powder was administered through diet to rats and obvious symptoms of toxicity and mortality were monitored for 72 h . Acute doses of GC seed powder did not induce any symptoms of toxicity or mortality of rats. In subchronic toxicity study, 1.0 – 10.0% of the GC powder was administered to rats through diet for 14 weeks. Dietary feeding of GC seed powder did not produce any mortality, no significant changes in food intake, gain in body weight, relative weight of organs, hematological parameters, macroscopic and microscopic changes in vital organs, were observed between experimental and control groups. Clinical enzymes viz., LDH, SGPT were within normal levels, however, the serum ALP and SGOT were significantly increased in male rats receiving 5.0 and 10 % of GC seeds. The results showed that acute and subchronic feeding of GC seed for 14 weeks did not produce any toxic effects in male and female rats and thus can be considered non-toxic and safe.

Industrial relevance: Herbal medicines are very popular and extensively used in the developing countries. Garden cress seeds and leaves are used in food preparations. GC seeds are given to pregnant and lactating women as natural food supplement to increase milk secretion. GC seeds are used in herbal based medicinal preparations. The data on the acute and subchronic toxicity studies on medicinal plants are essential to assess its safety to humans, particularly for its use in pharmaceutical preparations.

Key words: Garden cress seeds, acute toxicity, subchronic toxicity, albino rats

Introduction

Garden cress (*Lepidium sativum* Linn.) is an annual herb, belonging to Brassicaceae family. *Lepidium sativum* is a polymorphous species and believed to have originated primarily in the highland region of Ethiopia and Eritrea. Garden cress (GC) or pepper cress, is a fast-growing edible plant. Seeds, leaves and roots of GC are of economic importance, however, the crop is mainly cultivated for seeds. GC is also known as Asalio or Chandrasur in local languages and it is an important medicinal crop in India (Tiwari & Kulmi 2004). It is an erect, glabrous, annual, herbaceous plant growing upto 15-45cm in height. It has small white flowers in long recemes. The pods are obovate or broadly, elliptic, rotundate, emarginated, notched at apex and winged. It can be grown at all elevations, throughout the year, but the best crop is obtained in the winter season (Wealth of India, 1962). The leaves of the plant are consumed raw in salads, cooked with other vegetables and used to garnish food. Leaves are diuretic and gently stimulant (Maghrani et al 2005).

GC seeds are brownish red in colour and oval in shape. Morphologically, GC seeds resemble that of an oil seed with the dicotyledonous endosperm accounting to 80–85% of the seed matter, the seed coat account for 12–17% and the embryo for 2–3% of the seeds, respectively. Seeds contain 25 % of protein, 14-24 % of lipids, 33-54 % of carbohydrates and 8 % of crude fiber (Arkroyd et al 1966; Mathews et al 1993). The carbohydrates of the GC seeds comprise of 90.0 % non-starch polysaccharides and 10 % of starch. The seed bran has high dietary fibre content and also it has high water holding capacity. GC bran can be used as a rich source of dietary fibre (Gokavi et al 2004). GC seed contains 20-25% yellowish semidrying oil and the major fatty acid in it is alpha linolenic acid (32-34.0%) (Diwakar et al 2010). Garden cress oil (GCO) has a balanced amount of

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polyunsaturated fatty acids (PUFA) (46.8%) and monounsaturated fatty acids (MUFA (37.6%) and also contains natural antioxidants viz., tocopherols and carotenoids which protect the oil from rancidity (Diwakar et al 2010). Seven imidazole alkaloids, lepidine B, C, D, E, and F (dimeric) and two new monomeric alkaloids semilepidinoside A and B, sinapic acid and sinapin were reported in seeds of *L. sativum* (Maier et al 1998; Schultz & Gmelin, 1952). Benzyl isothiocyanate and benzyl cyanide were reported in colourless volatile oil, β-sitosterol and α-tocopherol were reported in the unsaponifiable matter of GC seeds (Lofty, 1957; Vasudev, 1956). A detailed study on physicochemical properties of garden cress seed oil has been reported (Diwakar et al 2010).

GC seeds have been used in traditional medicine since ancient times in India (Mali et al 2007). The seeds are aperients, diuretic, tonic, demulcent, aphrodisiac, rubefacient, carminative, galactagogue and emmenagogue (Nadkarni, 1954). It is supplemented in the diet of lactating women to increase the milk secretion during post natal period (Sahsrabudde & De, 1943) and recommended for diarrhea and dysentery (Kirtikar & Basu, 1981). The roots are bitter, acrid and are useful in treatment of secondary syphillis and tenesmus and used as a condiment (Uphof, 1959). The aqueous extract *of Lepidium sativum L*. seeds exhibits hypoglycaemic activity both in normal and diabetic rats without affecting insulin secretion (Eddouks et al 2005). GC seeds are shown to reduce the symptoms of asthma and improve lung function in asthmatics (Paranjape & Mehta 2006). GC seed mucilage is used as a substitute for gum Arabic and tragacanth (Patel et al 1987). GC seeds are widely used in many traditional medicinal preparations including cough syrups. However, there are few controversial reports in the literature regarding the safety of GC seeds for use as food and medicinal supplement. The present study was aimed at assessing the safety evaluation of Garden cress seeds by acute and subchronic toxicity studies in Wistar rats.

Materials and methods

Chemicals: Mineral mix, vitamin mix and cellulose were obtained from Sisco Research Laboratories, Mumbai, India. Rat diet was obtained from M/S Gold Mohar, Lipton India Ltd. Clincal enzyme kits such as serum glutamyl oxaloacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), urea and creatinine were purchased from Agappe Diagnostics Ltd. Kerala, India. All the solvents used were of analytical grade.

Plant material: Garden cress seeds (*Lepidium sativum)* obtained from a commercial supplier in Mysore. Garden cress seeds were germinated, the plant along with seeds were identified and authenticated at Horticulture Department, University of Agricultural Sciences, Bangalore. The seeds were sun dried and powdered using a laboratory grinder.

Preparation of diet: Commercial stock pellet diet from M/S Gold Mohar, Lipton India Ltd. India, was used in the study. The pellet diet was powdered and different concentrations of GC seed powder was thoroughly mixed with powdered stock diet. Diets were prepared every week and stored at 4^{o} C in walk in cooler facility.

Experimental animals: Adult male and female Wistar rats (OUTB—Wistar, IND-cft (2c) of 8 week old (230 g) were employed for the acute toxicity study and weaned young male and female rats were employed for the sub-acute toxicity studies. The animals were obtained from the stock colony of the Institute Animal House Facility. The animals were grouped by randomized design and were housed individually in stainless steel cages in a room with a relative humidity of 60–70%, temperature of 25 ± 2^{o}C and exposed to a light and dark cycle of 12 h duration. Animal experiments were carried out based on the ethical guidelines laid down by the committee for the purpose of control and supervision of experiments on animals by the Government of India, Ministry of Social Justice and Empowerment (Institute Animal Ethical Committee IAEC No.85/05). The animals were fed diet supplemented with GC seed powder *ad libitum*. Animals were given fresh diet daily and left over diets were weighed and discarded. Gain in body weight of the animals was recorded every week.

Acute toxicity study: Adult male and female rats (30 rats) were randomly divided into 5 groups of 6 animals in each group. Rats were fed with a graded dose of GC seed powder mixed with diet at levels of 0, 0.5, 1.0, 2.5 and 5.0 g/kg body weight on day one only. Prior to dosing, rats were fasted overnight. Rats were observed for onset of any immediate signs of toxicity and also during the observation period of three weeks for any delayed acute effects. All the animals were killed humanely under mild ether anesthesia after three weeks and selected vital organs were excised, blotted, weighed and processed for routine histological examination.

Subchronic toxicity study: Weaned rats of both sexes (30 rats) were randomly divided into 5 groups of 6 animals in each group. The rats were fed diets containing 0, 1.0, 2.5, 5.0, 10.0 % of GC seed powder (w/w) for a period of 90 days to assess the cumulative effects of GC seeds on Wistar rats. Daily feed intake and weekly body weights were monitored and all the animals were observed carefully for the onset of any signs of toxicity. At the end of experimental period all the animals were killed humanely under light ether anesthesia.

Organ weight and histopathological studies: The vital organs of each rat such as liver, lungs, kidney, spleen, brain, adrenals, gonads and heart were excised, blotted weighed and the organ/body weight per 100g ratio were calculated. A portion of the organ was fixed in Bouin's fixative. The organs were embedded in paraffin and 5 microns thick sections were cut and stained with hematoxylin and eosin for microscopic examination.

Specimen collection and processing: Blood samples were collected for hematological studies in dry test tubes containing sodium citrate as anticoagulant. A portion of the blood was collected without anticoagulant and allowed to clot at room temperature. Serum was separated by centrifugation at 3000g for 10 min and stored at minus 80^{o} C until analysis.

Hematological study: Red blood cells (RBC) and white blood cells (WBC) were counted in a hemocytometer. Hemoglobin concentration was estimated by reacting with Drabkins' reagent. Mean corpuscular hemoglobin (MCH) was calculated from hemoglobin and erythrocytes counts, whereas mean corpuscular hemoglobin concentration (MCHC) was determined from hemoglobin concentration and packed cell volume values (Bharoucha, 1976). Blood smears were made on glass slides and stained with Leishman for differential count (DC) of WBCs.

Assay of clinical enzymes: Activities of serum glutamyl oxaloacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT), serum alkaline phosphatase (ALP), serum lactate dehydrogenase (LDH), blood urea and creatinine were estimated using commercially available enzyme kits (Agappe Diagnostics Ltd. Kerala, India. The enzyme activities were expressed as U/L.

Statistical analysis: Results were expressed as mean ± standard error of mean (S.E.M.) Data on weekly body weights, food intake, relative organ weights and biochemical and hematological determinations were analyzed by student's t-test. A probability level of $p < 0.05$ was considered significant.

Results

Acute toxicity studies: Rats fed on acute doses of GC seeds did not develop any clinical signs of toxicity or death either immediately or during the post treatment even at highest dose of 5.0 g/kg body weight. The food intake in both sexes of treatment groups were comparable to that of control group. The relative organ weights of brain, liver, lungs, heart, kidney, spleen and testis/ovary of treated groups were comparable to that of controls (Table 1 & 2). No macroscopic or microscopic alterations were observed in internal organs in any of the treatment groups of GC seeds.

Table 1. Effect of dietary feeding of acute doses of GC seeds on relative organ weight of male rats

Organ weight (g/100g b.wt.)	Control	GC seeds 0.5 g/kg b.wt.	GC seeds 1.0 g/kg b.wt.	GC seeds 2.5 g/kg b.wt.	GC seeds 5.0 g/kg b.wt.
Liver	3.40 ± 0.54	3.17 ± 0.33	3.47 ± 0.58	3.70 ± 0.7	3.53 ± 0.7
Lungs	0.58 ± 0.14	0.56 ± 0.11	0.55 ± 0.05	0.58 ± 0.09	0.59 ± 0.13
Kidney	0.76 ± 0.09	0.69 ± 0.03	0.73 ± 0.07	0.73 ± 0.01	0.76 ± 0.09
Heart	0.32 ± 0.07	0.35 ± 0.02	0.35 ± 0.04	0.33 ± 0.01	0.32 ± 0.02
Spleen	0.33 ± 0.01	0.27 ± 0.01	0.24 ± 0.02	0.17 ± 0.09	0.24 ± 0.05
Testis	1.34 ± 0.17	1.16 ± 0.21	1.27 ± 0.03	1.25 ± 0.07	1.29 ± 0.18
Brain	0.69 ± 0.09	0.72 ± 0.02	0.67 ± 0.12	0.73 ± 0.04	0.68 ± 0.09

Values are mean ± S.E.M. of six animals. No significant difference between control and GC seed fed rats.

Table 2. Effect of dietary feeding of acute doses of GC seeds on relative organ weight of female rats

Organ weight (g/100g b.wt.)	Control	GC seeds 0.5 g/kg b.wt.	GC seeds 1.0 g/kg b.wt.	GC seed2.5 g/kg b.wt.	GC seeds 5.0 g/kg b.wt.
Liver	3.24 ± 0.35	3.44 ± 0.55	2.90 ± 0.18	2.93 ± 0.35	2.82 ± 0.19
Lungs	0.60 ± 0.07	0.62 ± 0.07	0.55 ± 0.05	0.55 ± 0.06	0.54 ± 0.01
Kidney	0.67 ± 0.05	0.82 ± 0.32	0.59 ± 0.05	0.63 ± 0.05	0.61 ± 0.03
Heart	0.34 ± 0.03	0.40 ± 0.07	0.32 ± 0.02	0.34 ± 0.04	0.34 ± 0.02
Spleen	0.26 ± 0.03	0.29 ± 0.07	0.25 ± 0.02	0.24 ± 0.02	0.23 ± 0.02
Testis	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Brain	0.71 ± 0.31	0.87 ± 0.07	0.81 ± 0.09	0.80 ± 0.06	0.79 ± 0.03

Values are mean ± S.E.M. of six animals. No significant difference between control and GC seed fed rats.

Subchronic toxicity studies: Dietary feeding of low doses of GC seed powder for 14 weeks did not produce any obvious signs of toxicity or mortality during the experimental period at any of the dosage levels of GC seeds. The food intake, in both sexes of treatment groups were comparable to that of control group. In general, incorporation of GC seed powder even at 10% level (w/w) did not affect the food intake in both sexes of treatment groups and were comparable to that of control group. The food intake, mean body weight gain and intake of GC seed/kg b.wt/ rat/ week are presented under Tables 3 & 4. No significant differences in body weight gain was observed in treatment groups of both sexes during 14 week experimental period. There were no

marked differences in the group mean relative weights of various vital organs in the GC seeds fed rats compared to that of control group (Tables 5 & 6).

Data on the various hematological parameters are presented under Tables 7 & 8. The mean values of hematological parameters such as RBC counts, WBC counts, hemoglobin content, packed cell volume, and differential counts of were essentially identical between control and GC seed fed groups.

Gross examination of vital organs during autopsy did not reveal any abnormalities that could be attributed to GC seed powder feeding in both sexes of rats. Further on microscopic examination, no treatment related histopathological alterations were observed in any of the vital organs irrespective of GC seed powder concentration. Detailed histological examination of each vital organ was as follows: Liver was characterized by normal hepatic cells with distinct nuclei and normal eosinophilic cytoplasm with normal sinusoids. Kidney displayed normal renal architecture with normal glomerules, proximal tubules and collecting ducts. Adrenals showed normal layer of cells in both cortex and medulla. Lung tissue showed fine alveolar sac structures and well defined alveolar ducts. Normal appearance of heart muscle was observed. Spleen showed normal architecture with well defined follicles, containing germinal centers. Brain showed normal cellular architecture both in cerebrum and cerebellar regions. Testis showed normal seminiferous tubules showing different stages of germinal cells, with spermatozoa. Ovarian tissue showed different stages of follicular development with mature corpus leuteum.

Table 5. Food intake, body weight gain and GC seed powder intake by male rats fed with GC seeds for 14 weeks

Duration of feeding (weeks)	Control		1% of GC seed powder			2.5% of GC seed powder			5.0% of GC seed powder			10.0 % of GC seed powder		
	Food intake (g/rat/ day)	Body weight (g/rat/ week)	Food intake (g/rat/ day)	Body weight (g/rat/ week)	GC seed intake (g/kg b.wt/ rat/ week)	Food intake (g/rat/ day)	Body weight (g/rat/ week)	GC seed intake (g/ kg b.wt/ rat/ week)	Food intake (g/rat/ day)	Body weight (g/rat/ week)	GC seed intake (g/kg b.wt/ rat/ week)	Food intake (g/rat/ day)	Body weight (g/rat/ week)	GC seed intake (g/kg b.wt/ rat/ week)
1	6.2	34.6	6.6	42.6	1.54	6.2	45.5	3.40	6.6	42.1	7.83	6.5	44.9	14.5
2	6.2	60.2	8.5	69.3	1.22	9.4	69.9	3.30	7.7	66.8	5.76	6.7	64.5	10.4
3	8.0	98.9	9.2	108.1	0.85	9.2	108.9	2.10	8.0	106.7	3.74	7.5	106.7	7.02
4	7.9	125.7	8.8	129.6	0.67	8.3	129.7	1.60	7.4	128.0	2.89	7.7	128.0	5.01
5	7.8	162.9	9.6	164.3	0.58	8.4	171.4	1.22	8.1	165.5	2.44	7.8	165.5	4.71
6	9.5	180.5	9.8	183.3	0.53	9.6	188.4	1.27	9.8	181.5	2.69	8.9	183.5	4.85
7	9.1	212.6	9.8	209.0	0.47	9.7	194.8	1.03	9.7	212.5	2.28	9.1	218.5	4.16
8	9.2	231.5	10.0	226.5	0.44	9.7	234.5	1.03	9.7	234.2	1.84	9.3	238.2	3.90
9	9.6	260.3	11.5	247.5	0.46	11.1	257.0	1.07	11.0	263.5	2.08	10.4	270.3	3.84
10	10.6	272.2	12.0	259.2	0.46	11.5	265.5	1.08	11.4	276.0	2.06	11.1	280.9	3.95
11	12.0	272.9	12.0	269.3	0.44	12.0	285.0	1.05	12.0	284.2	2.11	11.8	301.7	3.91
12	11.5	298.5	11.7	278.4	0.42	10.9	290.3	0.94	11.9	293.8	2.03	11.6	310.8	4.32
13	12.0	301.3	13.5	282.2	0.47	14.1	291.5	1.20	13.3	294.5	2.25	13.2	311.0	4.24
14	13.3	301.0	14.6	282.0	0.51	15.0	295.3	1.26	14.0	305.7	2.28	14.1	315.5	4.46

Values are mean ± S.E.M. of six animals. No significant difference in food intake and body weight was found between control and GC seed fed groups.

Table 6. Food intake, body weight gain and GC seed powder intake by female rats fed with GC seeds for 14 weeks

Duration of feeding (weeks)	Control		1% of GC seed powder			2.5% of GC seed powder			5.0% of GC seed powder			10.0 % of GC seed powder		
	Food intake (g/rat/ day)	Body weight (g/rat/ week)	Food intake (g/rat/ day)	Body weight (g/rat/ week)	GC seed intake (g/kg b.wt/ rat/ week)	Food intake (g/rat/ day)	Body weight (g/rat/ week)	GC seed intake (g/ kg b.wt/ rat/ week)	Food intake (g/rat/ day)	Body weight (g/rat/ week)	GC seed intake g/kg (b.wt/ rat/ week)	Food intake (g/rat/day)	Body weight (g/rat/ week)	GC seed intake (g/kg b.wt/ rat/ week)
1	6.8	43.9	7.2	44.0	1.64	6.9	44.4	3.88	7.1	42.8	8.29	6.5	46.7	13.9
2	7.6	66.6	8.3	68.9	1.20	8.1	66.9	3.00	6.4	66.2	5.00	6.7	69.4	9.65
3	8.5	94.8	9.4	98.0	0.96	9.2	97.0	2.37	8.2	95.4	3.80	7.5	100.5	7.46
4	8.9	104.4	9.3	110.8	0.83	9.2	109.3	2.10	8.7	107.8	4.03	7.7	114.2	6.74
5	9.1	123.2	9.4	128.3	0.73	9.2	128.4	1.79	8.1	124.8	3.24	7.8	136.8	5.38
6	9.6	132.3	9.8	141.3	0.69	9.9	136.6	1.81	8.1	133.8	3.03	8.9	144.8	6.15
7	8.7	139.0	9.7	151.8	0.59	9.8	148.2	1.65	8.9	144.8	2.84	9.1	155.3	5.85
8	8.7	146.8	9.7	162.0	0.67	9.6	156.2	1.54	8.2	156.5	2.62	9.3	162.1	5.73
9	9.6	152.2	11.6	172.3	0.59	10.9	170.2	1.52	9.8	156.8	3.03	10.4	170.6	6.09
10	10.4	159.6	11.0	184.9	0.60	11.5	180.5	1.59	10.5	169.7	3.09	11.1	179.3	6.19
11	10.5	165.6	11.5	189.0	0.62	10.6	182.4	1.45	11.0	171.2	3.21	11.8	183.9	6.41
12	11.0	165.8	12.0	191.7	0.65	12.0	184.7	1.63	12.5	173.9	3.59	11.6	186.8	6.20
13	12.0	171.2	12.8	196.5	0.65	12.4	183.4	1.69	12.5	182.0	3.71	13.2	189.7	6.95
14	13.2	171.4	13.8	181.7	0.76	13.6	189.3	1.79	13.8	185.5	3.72	14.1	190.9	7.30

Values are mean ± S.E.M. of six animals. No significant difference in food intake and body weight was found between control and GC seed fed groups.

Table 7. Effect of dietary feeding of GC seeds for 14 weeks on hematological profile of male rats

GC seeds g/100g diet	Hb (g/dl)	RBC (10^6/µl)	WBC (10^3/µl)	PCV (%)	MCV (pg)	MCH (%)	MCHC (%)	L	P	M	E
Control	13.62±0.48	9.20±0.51	8200±1219	40.2±0.75	44.17±2.58	15.00±1.28	33.84± 0.96	77.3± 1.38	21.0±1.0	1.7±0.50	1.2±0.01
1.0%	13.00±0.33	8.80±0.32	8800±1080	40.25±1.49	45.88±2.10	14.83±0.67	32.49±1.92	75.5± 2.22	23.0±2.0	2.0±0.70	1.1±0.02
2.50%	13.35±0.35	9.20±0.80	9412±1314	39.50±1.73	43.17±4.11	14.65±2.23	33.85± 2.44	72.0± 2.45	24.0±4.0	3.0±1.41	1.3±0.05
5.0%	12.60±0.45	9.20±0.51	10350±585	41.25±1.54	45.43±3.73	13.75±0.41	30.79± 2.27	71.3±1.31	26.0±1.0	2.0±0.40	1.3±0.02
10.0%	13.40±0.21	8.80±0.32	9950±1108	37.75±0.94	43.10±2.17	15.3 ±0.76	35.54± 0.79	73.3±2.29	25.0±2.0	1.0±0.20	2.0±0.0.3

Values are mean ± S.E.M. of six animals. Hb - hemoglobin, RBC-red blood cells, WBC - white blood cells, PCV - packed cell volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration, DC-differential count, L- lymphocytes, P- polymorph neutrophils, M -monocyte, E- eosinophils, B - basophils. No significant difference in hematological parameters were observed between control and GC seeds fed groups.

Table 8. Effect of dietary intake of GC seeds for 14 weeks on hematological profile of female rats

GC seeds g/100g diet	Hb (g/dl)	RBC (10^6/µl)	WBC (10^3/µl)	PCV (%)	MCV (pg)	MCH (%)	MCHC %	L	P	M	E
Control	13.47±0.48	8.60±0.60	10000 ±948	37.0 ±0.91	43.8±3.87	15.9±1.53	36.4±1.18	70.5±0.87	27.0±1	2.3±0.5	1.2±0.01
1.0%	13.37±0.67	8.40±0.51	11350±1252	36.5±1.32	44.0±3.42	16.1±1.62	36.8±2.36	74.3±0.75	24.0±1	2.0±0.5	1.0±0.01
2.50%	13.45±0.61	9.42±0.20	9300±685	36.2±2.78	38.7±3.69	14.2±0.60	38.1±4.41	72.3±1.38	25.0±1	1.8±0.4	1.2±0.02
5.0%	13.92±0.56	8.60±0.76	9900±1013	39.0±1.63	45.6±4.42	16.2±1.20	35.7±1.08	72.8±2.75	25.0±3	1.5±0.5	1.0±0.01
10.0%	13.95±0.55	9.20±0.40	9250±543	35.5±1.75	38.6±1.43	15.2±0.74	39.4±1.72	76.0±0.91	22.0±1	1.3±0.3	1.5±0.03

Values are mean ± S.E.M. of six animals. Hb - hemoglobin, RBC-red blood cells, WBC - white blood cells, PCV - packed cell volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration, DC-differential count, L- lymphocytes, P- polymorph neutrophils, M-monocyte, E- eosinophils, B - basophils. No significant difference in hematological parameters were observed between control and GC seeds fed groups

Table 9. Effect of dietary feeding of GC seeds for 14 weeks on serum enzymes and other biochemical parameters of male rats

GC seeds g/100g diet	LDH (U/L)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Creatinine (mg/dL)	Urea nitrogen (g/dL)
Control	568.8 ± 16.7	175.7± 34.4	62.8 ± 5.7	114.7 ± 58.5	0.70 ± 0.01	1.80 ± 0.07
1.0%	559.2 ± 45.2	170.6 ± 19.4	59.3 ± 9.0	111.7 ± 44.8	0.61 ± 0.02	1.95 ± 0.23
2.5%	525.3 ± 31.1	185.6 ± 34.5	71.5 ±12.9	143.5 ± 91.7	0.55 ± 0.01	1.85 ± 0.16
5.0%	570.0 ± 39.0	189.5 ± 26.9	88.2 ± 18.7*	247.6 ± 76.0*	0.59 ± 0.02	1.86 ± 0.21
10%	536.0 ± 24.3	176.1 ± 16.5	81.7 ± 5.0*	289.1 ± 56.2*	0.59 ± 0.01	1.93 ± 0.14

Values are mean ± S.E.M of six animals. * significantly different at <0.05 compared to control group

Table 10. Effect of dietary feeding of GC seeds for 14 weeks on serum enzymes other chemical parameters of female rats

GC seeds g/100g diet	LDH (U/L)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Creatinine (mg/dl)	Urea nitrogen(g/dl)
Control	437.0 ± 39.4	249.0 ± 48.7	94.0 ± 9.8	110.9 ± 5.2	0.58 ± 0.007	1.94 ± 0.11
1.0%	417.2 ± 73.0	212.6 ± 42.7	87.5 ± 30.7	105.9 ± 5.2	0.64 ± 0.031	1.53 ± 0.17
2.5%	422.2 ± 49.2	171.7 ± 36.0	58.1 ± 20.6	103.2 ±12.6	0.60 ± 0.031	1.54 ± 0.17
5.0%	408.5 ± 70.6	172.4 ± 11.5	58.1 ± 15.3	108.2 ± 29.3	0.53 ± 0.021	1.58 ± 0.11
10%	410.7± 88.6	163.6± 1.92	66.4 ± 13.1	103.3 ± 9.1	0.48 ± 0.017	1.44 ± 0.19

Values are mean ± S.E.M of six animals. No significant difference was observed in clinical enzymes, creatinine and urea levels between control and GC seed fed groups.

Data on various serum enzymes viz., serum lactate dehydrogenase (LDH), serum alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum urea and creatinine are represented in Tables 9 & 10. No marked alterations in serum enzymes namely LDH, SGOT and urea, creatinine levels were observed in GC seed powder fed male and female rats compared to that of control rats. However, a significant increase in serum ALP and SGPT was observed in male rats fed with 5 and 10 % of GC seeds in diet. The parameters remained within the normal range. This indicates that GC seeds has no adverse effect on liver and kidney functioning.

Discussion

Garden cress is an ancient herb that has been widely consumed and also used in traditional medicine. There are very few reports in the literature regarding the safety of GC seeds for use as food. A toxicity study on seeds of *L. sativum* in Wistar albino rats for 6 weeks was found to be non-toxic at 2%, but toxic at 10% (w/w) (Adam 1999). Similarly, Bafeel & Ali 2009 reported liver toxicity in rats fed with suspension of *L. sativum* seed extract at 2.0, 4.0 and 8.0 g/100/ mL. They observed significant decrease in body weight, histological changes in liver and increase in serum ALP, ALT levels after 6 weeks of feeding of *L.sativum* seed suspension at 4 and 8 g/kg b.wt. or 100, 200 and 400 mg/kg b.wt. However, our results showed that GC seeds were well tolerated without overt evidence of adverse effects at any of the doses administered. There were some fluctuations in food consumption and these differences were uniform between control and experimental groups. Terminal body and organ weight values were unaffected by feeding GC seeds, macroscopic and microscopic findings were found to be normal. Serum ALP and SGPT levels were elevated in rats fed with high doses of GC seeds (5 and 10 %) in male rats. However, SGOT levels were found to be lower in female rats. Similar results were reported in albino rats fed with high doses of GC seeds (Adam 1999; Bafeel & Ali 2009). The varied biological responses of GC seeds in above cited studies could be due to the difference in the route of administration, suspension of seed extract and short term feeding (3 to 6 weeks) of high doses GC seeds than the doses used in this study. Further, GC seeds did not induce microscopic changes in the rat liver, hence the elevated serum ALP and SGPT levels cannot be attributed to the toxicity of seeds. Isothiocyanates present in GC seeds (Virtanen & Saarivirta 1962; Burow et al 2007) could be responsible for high ALP and SGPT levels in rats. Therefore, the changes observed in serum chemistry may not be of toxicological significance as isothiocyanates are beneficial compounds present in Cruciferae family which are shown to be potent inducers of phase II detoxification enzymes involved in metabolism of xenobiotics and also chemical carcinogens (Munday and Munday, 2004).

In conclusion, the results of the present study clearly showed that the GC seeds under the conditions tested, did not induce acute or subchronic toxic effects in Wistar rats. GC seed was well tolerated and did not induce toxic effects even at 10% dietary level as evidenced by absence of any ill effects on growth, body weight gain, organ weight, histology, hematology or clinical enzymes in Wistar rats.

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