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A Pilot Study: The Effects of an Aqueous Extract of *Lepidium sativum* Seeds on Levels of Immune Cells and Body and Organs Weights in Mice

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ABSTRACT

Garden cress (*Lepidium sativum*, LS) seeds are used in many different parts of the world for different conditions and ills. Therefore, it was the aim of this study to determine the effects of an aqueous LS seeds extract on the immune system and general health in mice. This represents the first research study on the effects of LS seeds in mice. An aqueous extract of ground LS seeds was orally gavaged to young adult male Swiss Albino mice at a low dose (LD, 0.5 ml, 4 mice) and a high dose (HD, 1 ml, 4 mice) daily for 19-21 days, while control mice (2 mice) received saline solution by oral gavage. Whole blood was collected for a differential complete blood count. Body weights were measured every three days, and the liver, kidneys, spleen, and the combined lungs, heart and thymus were harvested and weighed. Results showed that, compared to the control, there were statistically significant increases in the mean white blood cell count and mean spleen weight for the LD group, while for the HD group the increases tended to significance. The mean body weight for the HD group showed clear increases compared to the control. Mean white blood cell types, red blood cell, and platelet counts; mean hemoglobin concentration; mean total body weight gains; and weights of the organs, except for the spleen, were not significantly different for the LD and HD groups compared to the control. Therefore, it may be concluded that the extract leads to some enhancement of the immune system. It is recommended that further research work may be carried on different concentrations of the extract using additional parameters.

Keywords: Garden cress, Mice, Red blood cells, Hemoglobin, Platelets, White blood cells.

INTRODUCTION

Many herbs are used extensively in traditional, alternative, herbal, or folk medicine in many different parts of the world, in addition to their use in food and fragrances. Many of the currently used pharmaceutical medicines were derived from plants and are based on their traditional uses. Some of the more commonly used herbs are cinnamon, turmeric, ginseng, ginger, and nigella sativa, which have many different effects including antioxidant and anticancer activities. A commonly used herb in Middle Eastern and Asian countries for the treatment of many diseases and conditions is the garden cress plant and its seeds.

Garden cress (*Lepidium sativum L.*, LS) belongs to the Brassicaceae or Cruciferae family of the plant kingdom that includes cruciferous vegetables such as cauliflower, cabbage, broccoli, sprouts and green leafy vegetables. Garden cress is an annual polymorphous species with edible seeds, leaves and stems.[1] It is widely used in the Middle East in folk medicine and in India in ayurvedic medicine, and it is grown in many parts of the world, including Saudi Arabia, Europe, Africa, and the United States of America. The seeds are said to have thermogenic, depurative, rubefacient, tonic, abortive, ophthalmic, diuretic, contraceptive, and anti-cancer activities.[1-4] They are also used for bone healing[5,6], reducing inflammation, cough (bronchial asthma)[7] and anemia.[1,4,8] It is essential that the seeds are used at the recommended therapeutic doses and for the appropriate length of time since the seeds contain substances with known toxicities at high doses, although there is no consensus on the safe doses.[8,9]

Lepidium sativum seeds contain many nutrients and phytochemicals with a wide range of important biological functions.[1,4] Seeds contain ω-6 (n-6) and ω-3 (n-3) essential fatty acids, which are important for health. They also contain sterols and glucosinolates, which are major secondary metabolites and are responsible for the distinctive sharp and bitter taste of the seeds. The seeds also contain benzyl thiocyanate and benzyl isothiocyanate, which are produced from glucotropaeolin, which is the main glucosinolate in the seeds.[1,4]

A main interest for us is the anti-cancer or chemopreventive activity of the aqueous LS seeds extract. The

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extract was previously used against cancer cells in vitro and it provided clear evidence of preferential killing of human cancer cells compared to non-cancer human cells.[2,3] It is well known that the immune system is sometimes able to fight and kill cancer cells. Thus, substances that are able to enhance the immune system non-specifically may also lead to the immune system becoming more able to fight and kill cancer cells.

Most studies on LS seeds have been done using alcoholic extracts or using powdered or whole seeds mixed with either drinking water or feed.[1,4] In addition, different studies use various amounts and preparations of LS seeds. After extensive searches on the net, only two studies on the effects of LS seeds on immune parameters were found although only one was *in vivo*. Diwakar, Lokesh and Naidu [10] found that LS seeds oil increased the *ex vivo* production of some inflammatory mediators by peritoneal macrophages of rats. The study by Datta *et al*. [9] showed that administration of LS seed powder to rats in their diets did not lead to any changes in body weights, organ weights, nor hematological parameters. Therefore, it is the aim of this research to study the effects of an aqueous LS seeds extract on body weight, some vital organs weights, and some hematological parameters that are linked to the immune system and general health. The findings may help in determining whether the LS extract influences the immune system positively thereby leading to the anti-cancer effects observed in our previous research findings.

MATERIALS AND METHODS

Preparation of the aqueous LS seeds extract

LS seeds were obtained from a local herbs shop in Jeddah, Saudi Arabia. The type obtained was grown in Al-Qaseem region of Saudi Arabia, as indicated by the herbalist. The seeds and the grown seedlings from the seeds were confirmed to be LS seeds by a taxonomist.

The method used for the extraction of LS seeds was based on Moroccan and local traditional methods of extraction and consumption of LS seeds and it was also used in previous research work.[2,3] LS seeds were ground to a coarse powder in an electric grinder, to allow for the conversion of the glucotropaeolin to benzyl isothiocyanate. Exactly 1g of powdered seeds was mixed with 100 ml deionized water and boiled for 10 minutes, after which the extract was left to cool for 15 minutes at room temperature**.** The extract was then filtrated by sterile gauze using a Buchner funnel to remove the gel-like substance and the ground seeds. The extract was stored at -80°C until further use and it was used for ten days only.

Experimental animals

A total of 10 young adult male Swiss albino mice, with an initial body weight of 21–43 g and about 4-6 weeks of age, were used in this study. All mice were supplied by and housed at King Fahd Medical Research Center, Jeddah, Saudi Arabia. The mice were housed at room temperature (25°C), and exposed to artificial lighting during working hours and to the natural light-dark cycle from windows for the remaining time. They were allowed free access to food and water during the entire experimental period. All mice received laboratory animal feed pellets (Grain Silos and Flour Mills Organization, Jeddah, Saudi Arabia), which contained 6% ash, 20% crude protein, 4% crude fat, 3.50% crude fiber, 1% calcium, 0.60% phosphorus, 20 IU/g vitamin A, 2.20 IU/g vitamin D, 70 IU/kg vitamin E and 2850 Kcal/kg energy.

The mice were randomly divided into 3 groups. Mice in the control group (2 mice) were administered with saline solution via oral gavage. The low dose (LD) group (4 mice) was administered with 0.5 ml and the high dose (HD) group (4 mice) was given 1 ml of the LS extract, both via oral gavage.

The experimental period ranged from 19 to 21 days depending on the group and the procedure done, as detailed below. All mice were individually weighed three days before the start of the experiment, at the beginning of the study, and every three days throughout the experimental period.

Blood and organs collection

Blood was collected from two mice from each group on the 21 st day of the experiment after an overnight fast. The mice took their usual doses the day before blood collection, thus they all took 20 doses. Whole blood was collected from the mice through the orbital sinus, while under diethyl ether anesthesia, in ethylene diamine tetra-acetic acid (EDTA) vacutainer tubes (Nassim Al-Safsaf, Vacutest Kima, Arzergrande, Italy).

Mice were dissected under anesthesia and the liver, kidneys, spleen, lungs, thymus, and heart were excised and weighed. Both kidneys of each mouse were weighed and the mean of the weights was used. Two of the LD mice were dissected on the 20 th day and the remaining two were dissected on the 21 st day of the experimental period. The two mice of the control group and two mice from the HD group were dissected on the 23 rd day of the experimental period.

Determination of differential complete blood counts

Hemoglobin concentrations, and total and differential white blood cells, red blood cell, and platelet counts were done at Al Borg Medical Laboratory, Jeddah, Saudi Arabia. All counts were done manually using a hemocytometer (Celeromics Company Cambridge, UK) and appropriate stains. For the determination of hemoglobin concentration, the Drabkin's cyanmethemoglobin reagent was used.

Descriptive and analytical statistics

All the reported data were recorded and graphically represented using the MegaStat statistical program, version 9.4, which was also employed for all statistical calculations. The following statistical results were calculated for all parameters: mean ($\overline{x}$), standard deviation (± SD), and standard error of the mean (± SE). After testing for the normal distribution and the homogeneity of the data, it was found that all the data follow the normal distribution. Therefore, the t-test was used to test for the significance in the differences between each LS group and the control. The resulting P values demonstrate significance or lack thereof as follows: $P > 0.05$ is a none significant (NS) difference, $0.01 \leq P \leq 0.05$ is a significant (S) difference, and $P < 0.01$ is a highly significant (HS) difference.

RESULTS

Extract preparation and yield percentages

Table 1 shows the initial volume of deionized water used for the preparation of the extract, mixed with the specified amount of LS, and the volume of the produced extract. The average percent yield was 50.2%. The volumes of extract obtained for the last times the extract was prepared were larger than the previous times probably due to the optimization of the procedure and especially with the filtration of the extract. The filtration step was where variable amounts of the extract were lost due to the efficiency of the filtration. The seeds soaked up a large amount of the water and became gel-like, therefore, it was challenging to filter as much extract as possible.

Table 1: Initial water volumes used and final extract volumes obtained in the extract preparation and yield percentages

Initial water volume (ml)	Weight of LS seeds powder (g)	Extract volume (ml)	Percent yield
100	1	52.5	52.5
400	4	141	35.3
500	5	230	46.0
500	5	163	32.6
500	5	216	43.2
500	5	372	74.4
500	5	338	67.6
Average			50.2

Determination of differential complete blood counts

Tables 2 and 3 show the effects of the LD and HD of the extract on total and differential WBCs, red blood cell, and platelet counts; and hemoglobin concentrations compared to the control groups. The t-test shows that there was a significant increase in the mean WBCs count for the LD group compared to the control group. As for the mean WBC types, RBC, and platelets counts; and mean hemoglobin concentrations, there were no significant differences between the LD and control groups. For the HD group, using the t-test, the mean total and differential WBCs, RBC, and platelet counts; and mean hemoglobin concentration were not significantly different from the control group.

Table 2: Descriptive statistics and test of significance, using the t-test, for the LD and control groups for the differential complete blood counts

Parameter	Group	Minimum	Maximum	Mean	±SD	±SE	Pvalue
WBC	Control	4.30	5.30	4.80	0.71	0.50	
($x10^3$cell/l)	LD	5.60	9.40	8.07	1.41	0.58	0.023^{S}
Neutrophil	Control	50	55	53	4	3	
(%)	LD	40	58	51	4	2	0.743^{NS}
Lymphocyte	Control	40	45	43	4	3	
(%)	LD	33	42	38	3	1	0.154^{NS}
Monocyte	Control	8	8	8	0	0	
(%)	LD	7	9	9	1	1	0.644^{NS}
Eosinophil	Control	2	2	2	0	0	
(%)	LD	1	3	2	1	0	1.000^{NS}
Basophile	Control	0	0	0	0	0	
(%)	LD	0	1	0	0	0	0.604^{NS}
RBC	Control	8.05	8.65	8.35	0.42	0.30	
($x10^6$cell/l)	LD	6.77	8.63	7.65	0.66	0.27	0.221^{NS}
Hemoglobin	Control	14	15	14	1	1	
(g/dl)	LD	12	16	14	2	1	0.688^{NS}
Platelets	Control	678	1103	891	301	213	
(cell/l)	LD	633	1005	799	139	57	0.548^{NS}

S: significant (P ≤ 0.05), NS: not significant (P > 0.05)

Table 3: Descriptive statistics and test of significance, using the t-test, for the HD and control groups for the differential complete blood counts

Parameter	Group	Minimum	Maximum	Mean	±SD	±SE	P-value
WBC	Control	4.30	5.30	4.80	0.71	0.50	0.073^{NS}
($x10^3$cell/l)	HD	6.20	9.20	7.38	1.36	0.68	
Neutrophil	Control	50	55	53	4	3	0.062^{NS}
(%)	HD	40	49	44	4	2	
Lymphocyte	Control	40	45	43	4	3	0.413^{NS}
(%)	HD	41	50	46	4	2	
Monocyte	Control	8	8	8	0	0	0.745^{NS}
(%)	HD	7	9	8	5	2	
Eosinophil	Control	2	2	2	1	1	0.116^{NS}
(%)	HD	1	2	1	5	2	
Basophile	Control	0	0	0	0	0	0.541^{NS}
(%)	HD	0	1	0	1	0	
RBC	Control	8.05	8.65	8.35	0.42	0.30	0.105^{NS}
($x10^6$cell/l)	HD	7.65	8.09	7.87	0.18	0.09	
Hemoglobin	Control	14	15	14	1	1	0.449^{NS}
(g/dl)	HD	13	14	14	0	0	
Platelets	Control	678	1103	891	301	213	0.366^{NS}
(cell/l)	HD	507	807	719	142	71	

NS: not significant (P > 0.05)

Table 4: Descriptive statistics and test of significance, using the t-test, for the mean total body weight gains for the LD, HD and control groups

Group	Minimum	Maximum	Mean	±SD	±SE	P value
Control	5	6	6	1	1	-
LD	-3	10	2	4	1	0.298^{NS}
HD	-2	7	3	4	2	0.384^{NS}

NS: not significant (P > 0.05)

Mean daily body weights for the groups

Mean body weights for the mice in each group were calculated for the weights taken three days before the start of the experimental period, weights taken at the start of the experiment and for the weights taken every three days of the experimental period. The plot of these weights for all groups (Figure 1) shows that the mean weights increased modestly for the LD and HD groups. As for the control group, the mean body weights fluctuated except for the last weighing (day 21), which was much higher than the previous weighing (day 18).

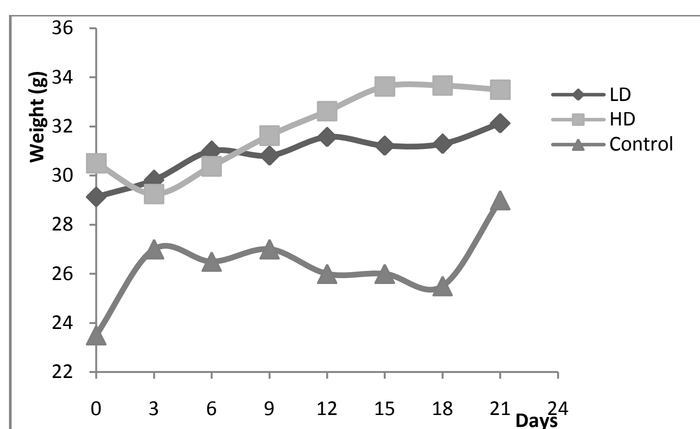

Figure 1: Mean daily body weights per group

Mean daily body weight gains for the groups

The body weight gain for each mouse for each weighing (every three days) during the experimental period was calculated by subtracting the previous body weight from the current body weight. Subsequently, the mean of the weight gains for the mice in each group was calculated. The plot for the mean weight gains for the groups (Figure 2) shows that the body weight gain per mouse per group varied between increasing and decreasing. Overall, the mean body weight gains for the control increased mainly in the second half of the experimental period. On the other hand, the mean body weight gains for the LD group increased slightly while the HD group showed the highest consistent increases for the body weight gains throughout the experimental period.

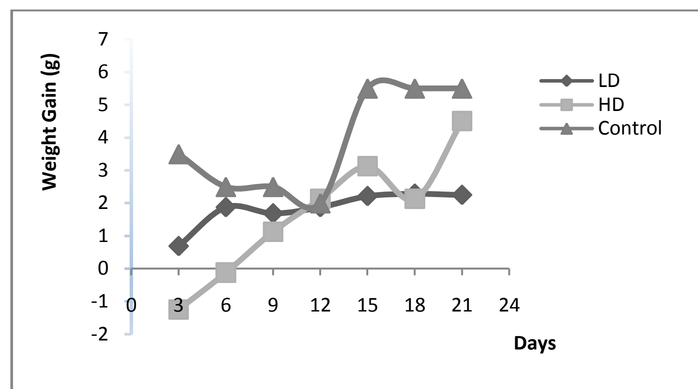

Figure 2: Mean daily body weight gains for the groups

Mean total body weight gains for the groups

The mean total weight gain for each mouse was calculated by subtracting the initial body weight for the mouse from its final body weight at the end of experimental period. The mean total body weight gain for each group was then calculated.

Table 4 shows the effect of the LS extract on the mean total weight gain of the mice compared to the control. At the end of the experimental period, the mean total weight gains of the mice in the LD and HD experimental groups were not significantly different, using the t-test, compared to the mean total weight gain for the control group.

Mean organs weights for the groups

The statistical results, using the t-test, for the mean organs' weights (Table 5) for the LD group compared to the control showed a significant increase of the mean spleen weight for the LD group compared to the control group. The mean liver, kidney, and lung-heart-thymus weights for the LD group were not significantly different from the respective mean weights for the control mice. The statistical results (Table 6), using the t-test, for the mean organs' weights for the HD and control groups showed no significant differences between the HD and control groups, although the mean spleen weight for the HD group tended to a significant increase compared to the mean weight for the control group.

Table 5: Descriptive statistics and test of significance, using the t-test, for mean organs weights for the LD and control groups

Organ	Group	Minimum	Maximum	Mean	±SD	±SE	P value
Liver	Control	1.76	1.86	1.81	0.07	0.05	0.819^{NS}
	LD	1.57	2.28	1.87	0.30	0.15	
Kidney	Control	0.30	0.32	0.31	0.01	0.01	0.784^{NS}
	LD	0.25	0.37	0.30	0.05	0.03	
Spleen	Control	0.11	0.13	0.12	0.01	0.01	0.044^{S}
	LD	0.18	0.28	0.22	0.04	0.02	
LHT	Control	0.47	0.52	0.50	0.04	0.03	0.327^{NS}
	LD	0.49	0.64	0.57	0.08	0.04	

LHT: lung, heart, and thymus, S: significant (P ≤ 0.05), NS: not significant (P > 0.05)

Table 6: Descriptive statistics and test of significance, using the t-test, for mean organs weights for the HD and control groups

Organ	Group	Minimum	Maximum	Mean	±SD	±SE	P-value
Liver	Control	1.76	1.86	1.81	0.07	0.05	0.244[NS]
	HD	1.90	2.13	2.02	0.16	0.12	
Kidney	Control	0.30	0.32	0.31	0.01	0.01	0.155[NS]
	HD	0.24	0.28	0.26	0.03	0.02	
Spleen	Control	0.11	0.13	0.12	0.01	0.01	0.053[NS]
	HD	0.30	0.42	0.36	0.08	0.06	
LHT	Control	0.47	0.52	0.50	0.04	0.03	0.126[NS]
	HD	0.56	0.61	0.59	0.04	0.02	

LHT: lung, heart, and thymus, NS: not significant (P > 0.05)

DISCUSSION

Herbal medicines have been used to treat different diseases in addition to offering the advantages of being easily available, cheap, and leading to few or no side effects. LS seeds are commonly used in many parts of the world for their beneficial activities and to treat some conditions and illnesses, although not much scientific evidence is available to validate these uses. The incidence of cancer has been increasing worldwide for the last several years. Therefore, it is important to discover chemopreventive agents that prevent or slow down carcinogenesis or that specifically kill cancer cells while sparing non-cancerous cells. It is sensible to try to find chemopreventive agents that are natural and thus easily available, cheap and with a few, if any, harmful side effects. Numerous research and epidemiological studies[2-4] have shown anticarcinogenic and chemopreventive effects of Cruciferous vegetables.

This study investigated the effects of oral administration of an aqueous extract of LS seeds on the immune system and general health in male Swiss albino mice. The extract was administered daily at two different volumes (0.5, and 1 ml) for 19-21 days. The effects of the extract were determined by measuring the differential complete blood counts, body weights of the mice, and weights of some major organs. The route of administration of the extract was by oral gavage since it introduces the extract directly into the gastrointestinal tract of mice, which resembles the normal ingestion of LS in humans and it ensures that the mice receive the specified amount daily.

The findings of this study showed no significant differences between the LD and HD groups each compared with the control for the mean neutrophil, lymphocyte, monocyte, eosinophil, basophile, RBC, and platelet counts; and hemoglobin concentrations. The mean WBC count for the LD group (8.07 ± 1.41) was significantly higher (P = 0.023) compared to the control group (4.80 ± 0.71). As for the HD group, the mean WBC count (7.38 ± 1.36) was not significantly different from the control, although it tended to a significant increase (P = 0.073).

The mean daily body weights for all groups increased as time passed from 3 days before the start of the experiment until the end of the experiment, with the control group showing greater increases compared to the LD and HD groups, which showed similar increases. Although if the mean weights for the weighing that was taken three days prior to the start of the experimental period is ignored, then the mean daily body weights for the HD group would show a much higher weight increase compared to the control and LD groups, while the LD and control groups will show comparable weight increases.

As for the mean daily body weight gains, the HD group showed a much higher overall total increase for the entire experimental period compared to the LD and control groups. The LD and control groups showed similar increases in the mean daily body weight gains. The results of the mean total body weight gains for the LD and HD groups did not show significant differences compared to the mean total body weight gain for the control group.

Therefore, it seems that the ingestion of the LD and HD of the extract lead to increases in the mean daily body weights and mean daily body weight gains compared to the control, although this is not reflected in the mean total body weight gains. This may be due to the fact that the control mice showed bigger weight increases in the last few days of the experimental period. Thus, a more detailed study of the weight changes of mice ingesting the extract should be carried to better clarify the weight gains effected by the extract.

The results of the mean liver, kidney, and mean combined lungs-heart-thymus weights for the LD and HD groups were not significantly different from the respective mean weights for the control mice. On the other hand, the mean spleen weight (0.22 ± 0.04) for the LD group was significantly higher (P = 0.044) and for the HD group (0.36 ± 0.08) it tended to a significant increase (P = 0.053) compared with the mean spleen weight for the control group (0.12 ± 0.01). The spleen and thymus were the only lymphoid organs that were weighed, although the weight of the thymus was not taken separately from the lungs and heart. Therefore, it is noteworthy that the spleen, an immune system organ, was the only organ that showed a difference (increase) from the control. In addition, the statistically significant increase was for the LD group only, and not the HD group, which showed clear and significant increases in the mean daily body weights and mean daily body weight gains compared to the control. Thus, it must be pointed out that this increase in the mean spleen weight for the LD group is not related to increases in the body weights of the mice in this group since they did not show notable increases compared to the control weights.

Therefore, these finding suggest that the ingestion of the LS aqueous extract enhances the immune system by increasing the weight of the spleen, which may result from the increased production and storage of immune system cells in the spleen. This is especially significant since the mean total body weight gain of the mice taking the extract did not increase significantly compared to the control mice. Providing further credence for the enhancement of the immune system is the observed significant increase in the mean WBC counts for the LD mice compared to the control, and the fact that also the mean WBC counts for the HD mice, although not significantly different, tended to a significant increase compared to the control. Therefore, it may be that the optimal extract dose for the enhancement of the immune system is the LD, while the HD is less effective.

The only research study that is similar to the current work is the work of Datta *et al.* [9], although they used seeds powder mixed with the regular diet of Wistar rats and they tested acute and chronic (for 14 days) doses. They did not find any significant differences in the mean hemoglobin concentration; and RBC, WBC, lymphocyte, neutrophil,

monocyte, eosinophil, and basophile counts compared to the control. Nor did they find any changes in food intake, gain in body weight, or relative weights of some vital organs. Thus, they concluded that the doses that they used were safe and non-toxic to the rats.

CONCLUSION

The aqueous LS seeds extract used in this research work might be useful for enhancing and strengthening the immune system. It is recommended that further work be done on different concentrations (or volumes) of the extract that are close to the LD volume used for longer time periods, in addition to measuring other immune-related parameters. A different concentration may lead to more pronounced enhancement of the immune system. It is hoped that the extract may be developed to be successfully used to enhance the immune system to help in preventing the formation of cancer and/or as an agent for killing cancer cells once they form. Some of the advantages the extract may offer are that it has no side effects, it is cheap, and readily available.

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