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EFFECTIVE USES OF ALMOND SEEDS IN IMPROVING IMMUNOLOGICAL AND HISTOPATHOLOGICAL FUNCTIONS IN HEPATIC RATS

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ABSTRACT

Immunity is the body's defense mechanism against invaders. Thus, in the event that infections manage to penetrate the skin or mucous membranes, the immune system acts as the body's natural defensive mechanism. This investigation sought to determine if almond seeds may benefit hepatic rats' immune and histological systems. In this study, the animal was kept in a cage during the trial. Prior to the trial, for a week, the rats were housed in groups of six and given a standard diet. The first set of rats were negative normal controls (C-ve) and were given nothing except a baseline diet for twenty-eight days. The survival rats (n=24) were inoculated with carbon tetrachloride (Ccl4). There were three groups given different amounts of (5%, 10%, 15% almond seed), Nonetheless, one group was given the disease diagnosis before the experimental diet was even given. The findings revealed mostly significant differences among all groups contrasted with the C+ ve group. But no significant differences were observed between G3, and G4 for Monocell Immune Distribution (MID) and Granulocytes (GRAN). The highest recovery was in rats fed 15% almond seed powder in (WBC), (LYM), (MID), (GRAN), (IgG) and (IgM). Different doses of almond seeds are indicated for boosting immunity in hepatic cases.

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Introduction

Having immunity means that your body can identify and get rid of harmful invaders. The immune system is the body's natural defense against foreign invaders that have already made their way through the skin or mucous membranes. It is sometimes referred to as the immunological response. However, the immune system weakens its ability to fight off disease as a reaction to the changes in hormones and metabolism that come with stress. These alterations may be traced back to the stress response [1]. Liver damage is a frequent disease that may develop into cirrhosis, fibrosis, chronic hepatitis, and finally hepatocellular cancer from steatosis. Since oxidative stress plays such a significant role in the onset and course of liver disease, antioxidants have been proposed as potential therapeutic agents and pharmaceutical adjuvants to guard against injury to the liver [2]. There are many different ways that plants can be used to treat illness, and traditional plant medicine is just one of them. Almond is a seed from a fruit that grows from an almond tree. The dense nutrition that almonds provide can benefit your body in several ways. If you want to improve your fertility, as well as the health of your heart and bones, you should try to do more exercise [3]. As long as you watch portion sizes, almonds, despite their high-calorie content, may help lower your risk of weight gain and obesity. Nuts' high protein and fiber content makes you feel fuller more quickly, allowing you to better regulate how many calories you eat while still feeling satisfied [4]. The almond is the most nutritious nut. Almonds are highly suggested as a nut for children due to their high protein and mineral content. These delectable nuts have a high concentration of vitamin E, a potent antioxidant that prevents cell damage from free radicals. Iron, which is crucial to maintaining a healthy immune system, is also abundant in brown nuts. A morning snack of 5-6 almonds, after they have been soaked and peeled, has been shown to improve immunity, lower inflammation, and lower the chance of acquiring chronic diseases [5, 6]. Indicates that frequent consumption of nuts may improve immunity and provide other health advantages. Consuming almonds regularly is a good

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idea because They are an excellent source of protein as well as fiber & micronutrients like vitamin E & iron. Giving you healthy fats & protein at the same time [7]. Reported that eating almonds helped their metabolism. In particular, the authors discovered that the study participants' resting energy expenditure increased due to a decrease in hunger and satiety when their diets included almonds. The consumption of almonds has been linked to a marginal but statistically significant decrease in both body weight & fat mass [8]. Demonstrated a link between magnesium consumption and decreased diabetes risk. This advantage could be provided by almonds, which contain magnesium. However, there is also data suggesting that eating almonds can help ward off diabetes [9]. Those who ingested 56 grams of almonds per day exhibited substantial improvements in immunity and lower levels of HbA1c, LDL cholesterol & total cholesterol in a twelve-week study of diabetic risk among young adults & adolescents.

Aim of the Study

The purpose of this research was to know the effective uses of almond seeds in improving immunological and histopathological functions in hepatic mice.

Materials and Methods

Materials

Preparation of Almond Seeds

The Al-Baha region's local market provided the almond seeds, which were then carefully cleaned, cut into little pieces, and dried at 50 degrees Celsius in an oven designated for drying for three days. Thereafter, they were crushed and processed into a powder.

Carbon Tetrachloride (Ccl4)

(Ccl4) was provided by El-Gomhoria Company for Chemical Industries in Cairo, Egypt, in the form of a liquid solution that contained ten percent of the substance. It was purportedly a toxic substance meant to cure liver illness and was marketed in one-liter white plastic water bottles. During induction, it was diluted with paraffin oil acquired from the pharmacy.

Experiential Animals

A total of 30 adult male Sprague Dawley adult rats (175-170 grams) were employed in this investigation, each one of them originated from the Medical Insects Research Institute in Doki, which is located in Cairo.

Rats were housed in wire cages for a week to adapt them to life in the lab while feeding them a standard laboratory meal. Both food and water were offered voluntarily and were subject to daily inspections; Water was supplied via glass tubes that were positioned against one side of the cage, and food was served in special dishes designed to stop spills.

Biological Experiment

Rats' Normal Diet

The nutritional breakdown of the basil diet was as follows: 10 percent casein, 0.25 percent choline chloride, 1 percent vitamin mixture, 5 percent cellulose, 10 percent maize oil, 4 percent salt mixture, 0.35 percent methionine & 69.5 percent corn starch [10].

In the experimental diet, the baseline had the following components: $MgSO_4.2H_2O$ (204 mg), $CaHPO_4.2H_2O$ (150 mg), $Fe(C_6H_5O_7)$ $26H_2O$ (55 mg), $ZnCl_2$ (0.5 mg), NaCl (334 mg), $CaCO_3$ (600 mg), $CuSO_4.5H_2O$ (0.06 mg), $MnSO_4.4H_2O$ (10 mg), K_2HPO_4 (645 mg), and Kl (1.6 mg) [11].

Test diet baseline components included Vitamin E (10 IU), Niacin (4.00 mg), Calcium Thiamin (0.50 mg), Vitamin K (0.50 IU), Folic acid (0.02 mg), Choline chloride (200 mg), pantothenic acid (0.40 mg), Calcium Thiamin (0.50 mg), Vitamin B12 (2.00 g), Para-amino-benzoic acid (0.02 mg), Vitamin A (200 IU), Pyridoxine (1.00mg), Inositol (24 mg), and Vitamin D (100 IU) [12].

Diet Experiment

The investigational food is shown in **Table 1** and is composed of a baseline diet supplemented with powdered plant matter at a ten percent rate.

Table 1. The experimental and basic diets' compositions.

Component (g)	Basal diet	5% Almond seeds	10% Almond seeds	15% Almond seeds
Test ingredients	---	5	10	15
Casein	20	20	20	20
Corn oil	4.7	4.7	4.7	4.7
Mineral mix	3.5	3.5	3.5	3.5
Vitamin mix	1	1	1	1

Cellulose	5	5	5	5
Choline chloride	2	2	2	2
Sucrose	10	10	10	10
Corn starch	Up to 100	Up to 100	Up to 100	Up to 100

Induction of Liver Intoxication in Rats

To induce long-lasting liver injury, adult male albino rats received twice-weekly intramuscular injections of (Ccl4) carbon tetrachloride mixed with fifty percent V/V paraffin oil (2 ml/kg B.W.T.). As a means of verifying the occurrence of liver injury & checking liver function after Ccl4 administration, specimens of the blood were taken using the retro-orbital approach.

Rats

Adult male Sprague-Dawley albino rats of the Sprague-Dawley strain, weighing 150–160 g at maturity (B.Wt). The animals spent 14-16 weeks in the Animal Laboratory before being moved. The animals were housed in neat, hygienic plastic cages covered in stainless steel. The rats were fed the baseline food for a week before to the experiment so they could become used to it. There was a small-mouth bottle with plastic tubing at the mouth that offered limitless access to water in addition to the metallic tube. As mentioned before, the rats had spent seven days becoming used to a twelve-hour light and dark cycles before the study started.

Experimental Design

- Every group of 6 rats was subdivided into 7 varied groups. There were several groups of rats:
- G_1: The "Control" group consisted of normal rats that were nourished a standard food for twenty-eight days lacking any further intervention.
- G_2: Toxic liver rats were kept as controls & given basic food for twenty-eight days with no additional therapy.
- G_3: Liver-intoxicated rats fed a base meal supplemented with five percent almond seed powder.
- G_4: Liver-toxic rats fed a base diet supplemented with ground almonds (10 percent in total).
- G_5: Liver-toxic rats fed a diet with fifteen percent almond seed powder added to their regular food.

Biological Evaluation

For the research's 28 days, participants recorded their daily food intake and weekly body weights. Organ weight, feeding efficiency ratio, and body weight growth (B.W.G.%) were all determined [13].

Blood Sampling

The participants in this research fasted for 12 hours before providing blood samples. To coagulate the blood samples, they were placed in a water bath at 37°C for a duration of half an hour after they were collected utilizing the retro-orbital technique by use of extremely refined glass tubing. Before the glucose test, blood samples were centrifuged for 5 min at 3000 rpm for serum extraction. After aspirating the residue, it was put in sterile polypropylene tubes with tight-fitting lids and frozen at -20^{o}C for analysis. After removal and washing in a salt solution, the liver, spleen, heart, & kidney were weighed & preserved in a 10 percent formalin solution as instructed by [14].

Immunologic Analysis

Lymphocyte Transformation Test. Lymphocytes [15]

The lymphocyte separation medium has thin layers of heparinized blood applied to its surfaces using sterile Pasteur pipettes. 50 ml Ficoll hypaque was filled in a sterile polystyrene centrifuge tube (1.1).

Phagocytosis and Killing Assay [16]

Leukocytes were primed for phagocytosis and destruction of germs by using blood collected in heparin-coated universal bottles. Red blood cells were lysed by treating the heparinized blood with 0.83 percent ammonium chloride, after which the samples were washed three times in phosphate buffer solution (PBS) that had a pH of 7.2 & a final suspension in MEM containing 0.55 percent deactivated serum derived from the foetal calf for a final concentration of ten PMN cells that are viable per milliliter.

Determination of (CRP)

The concentration was written as mg/dl at 370 c and the absorbance was set to 492 nm [15].

Histopathological Examination

The liver samples were taken right after the animals were killed when the experiment was done, then embedded in paraffin wax, washed with xylene, dried in ethyl alcohol, and fixed in ten percent neutral formalin. Slices with a thickness of 4-6 microns were stained using H&E [16].

Statistical Analysis

The statistics were derived from a one-way classification system. This is the least significant difference (LSD) between means from an analysis of variance (ANOVA) [17].

Results and Discussion

The purpose of our research was to know the effectiveness of using almond seeds in improving immunological and histopathological functions in hepatic rats.

Immunological Results

Effect of Different Levels of Almond Seeds Powder on WBC (k/ul)), LYM (%), MID, GRAN, IgG & IgM of Hepato-Intoxicated Rats

Data from **Table 2** indicated the impact of varying intensities of almond seed powder on Lymphocytes (LYM) (%), Minimum Inhibitory Dilution (MID), White Blood Cells (WBC) (k/ul), IgG, positive control, and IgM for the negative control, Granulocytes (GRAN) & other varied groups on hepatic rats.

It's obvious that in rats administrated by CC14 without therapy (C+ve group) mean values of (WBC), (LYM), (MID), (GRAN), (IgG) and (IgM) were low being (3.84±0.06 k/ul, 3.12±0.088%, 0.39±0.042%,0.12±0.02%, 384.39±5.06 GPL and 25.19±1.48 MPL, but through (C-ve group) normal rats were higher being; (10.39±0.34 k/ul, 5.84±0.11%, 1.92±0.15%, 1.33±0.055%, 867.5±9.84 GPL and 94.85±5.03 MPL), respectively. The data in the same **Table 2** revealed mostly significant differences between all groups as compared with the C+ ve group. However, no significant differences were observed between G3 and G4 for Monocell Immune Distribution (MID) and Granulocytes (GRAN). The highest recovery was in rats fed in 15% almond seeds powder in (WBC), (LYM), (MID), (GRAN), (IgG) and (IgM) being; (5.93±0.11k/ul, 5.2l± 0.63%, 1.41 ±0.07, 0.52±0.13, 954.09±5.29 and 00.02±2.05) respectively. In the latter best group recorded for G4 10% almond seeds powder. The data agree with [7]. They showed that polysaccharides of almond seeds have strong immune-modulating capabilities that boost monocyte and macrophage performance; results imply that almond seed polysaccharides have therapeutic potential at least in part due to their ability to explain the possessing therapeutic value of almond seed extracts (which involved primarily galacturonic acid, xylose, galactose, arabinose & rhamnose). Also the data agreement with [9]. They investigated specific immunomodulation of almond seeds on mice (body fluid and cells), and estimated that there was a significant increase of intravenous IgM & IgG levels, notably increasing T and B lymphocyte proliferation and bringing it back to a normal level. They said that nuts and seeds are full of powerful immune-boosting nutrients that can help to regulate blood sugar, prevent many chronic diseases, and even give your immune system a boost. In a pandemic time, there is no harm in getting a bit more nutrients into your diet more often. You can always be certain that we provide any type you require, from organic to roasted, salted, unsalted, and even candied versions.

Table 2. Effect of different levels of almond seeds powder on WBC (k/ul), LYM (%), MID, GRAN, IgG and IgM of hepatic rats.

Groups	Parameters (Liver)											
	WBC (k/ul)	**% change of C (+)**	**LYM (%)**	**% change of C (+)**	**MID (%)**	**% change of C (+)**	**GRAN (%)**	**% chang of C (+)**	**IgG (GPL)**	**% change of C (+)**	**IgM (MPL)**	**% change of C (+)**
G1 C (-)	I0.39±0.34 [a]	+ 170.57	5.84±0.11 [a]	+ 87.18	1.92±0.1 5 [a]	+392.31	1.33±0.05 [a]	+ 1008.3	867.5±9.84 [a]	+ 125.68	94.85±5.03 [a]	+276.54
G2 C (+)	3.84±0.06 [f]	--	3.12±0.088 [f]	--	0.39±0.042 [f]	-	0.12± [f]0.02	--	384.39±5.06 [f]	-	25.19±1.48 [f]	-
G3: almond seeds Powder 5%	5.11±0.28 [d]	+33.07	4.07d±0.23 [d]	+30.45	0.88±0.03 [d]	+ 125.64	0.33±0.04 [d]	+ 175	53l.12±11.39 [d]	+38.17	29.63±1.22 [d]	+ 17.63

G4: almond seeds Powder 10%	5.52±0.09 ^{c}	+43.75	4.62±0.05 ^{c}	+48.08	1.0l±0.22 ^{c}	+158.97	0.4 l± 0.08 ^{c}	+241.7	760.02±4.34 ^{c}	+97.72	92.51±1.79 ^{b}	+267.25
G5: almond seeds Powder 15%	5.93±0.1 ^{b}	+54.43	5.2l±0.36 ^{b}	+66.99	1.41±0.07 ^{c}	+261.54	0.52±0.13 ^{c}	+333.33	954.09±5.29 ^{b}	+ 148.21	I00.02±2.05 ^{c}	+297.06
LSD	0.358		0.334		0.216		0.169		14.500		5.043	

• Values denote arithmetic±standard deviation of the mean.
• Means in the same column with various letters are significantly different ($p < 0.05$).
• One-way ANOVA test used.

Histopathological Results

Liver

Liver sections from control rats had normal histological structure, as seen in the veins and portal area of the liver **(Figure 1)**. In contrast, both the liver and testes of the control-positive rats displayed obvious changes. Vacuolar degeneration, necrosis of hepatic cells, nuclear pyknosis, edema, and little infiltration of inflammatory cells in the portal region were all observed in the livers of control-positive rats **(Figure 2)**.

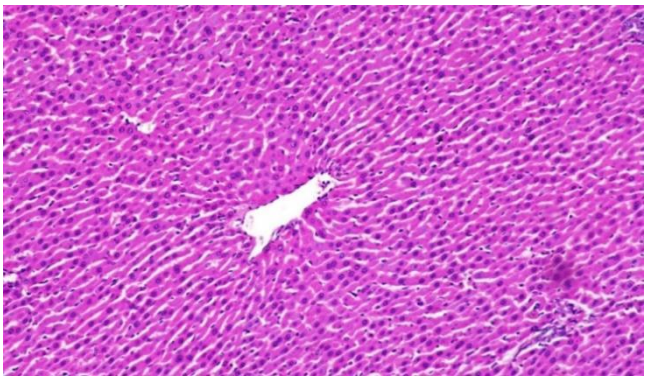

Figure 1. rat's Liver from the control (+) group showed typical histological structure of the hepatic cells, central vein & portal area (H and E × 200)

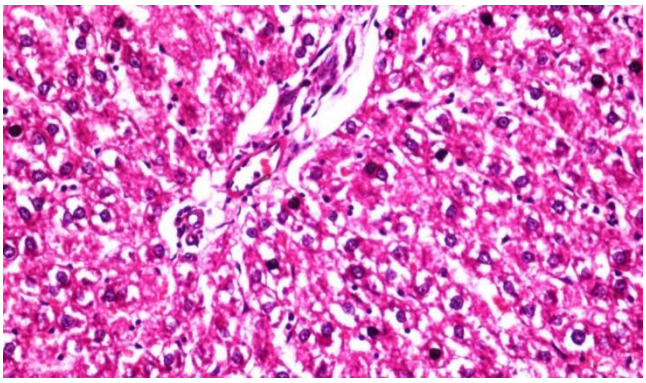

Figure 2. Rat liver with CCL4 poisoning that is not being treated (control +ve group) showing necrosis of the hepatic cells, marked vacuolar degeneration & nuclear pyknosis with edema & few inflammatory cells infiltration in the portal area. (H and E x 200)

Concerning the Controlled Subjects, the treatment of (5% almond seeds powder (showed pronounced safety for both hepatic and testicular tissue, while the use of (10% almond seeds) showed a protective effect for the hepatic tissue but no obvious protection to the testicular tissue. Livers of control-positive rats administrated with (5%, 10% and 15% almond seeds) had considerable hepatocellular deterioration and scattered necrosis but did an excellent job of protecting the hepatic parenchyma **(Figures 3-5)**.

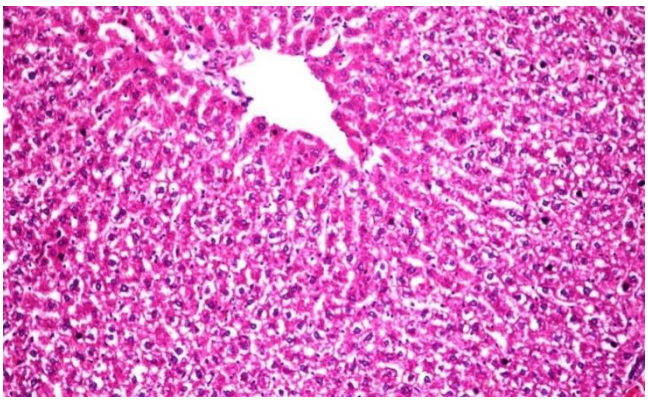

Figure 3. Rat liver poisoned with CCL4 given 5% powdered almond seeds showing marked protection of both hepatic and testicular tissue (H and E x200)

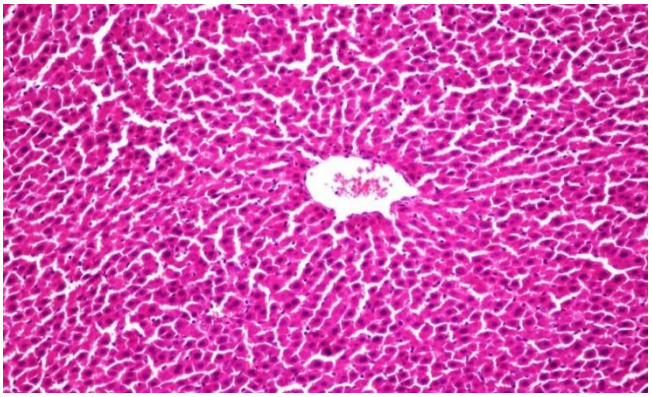

Figure 4. Rat liver poisoned with CCL4 given 10% powdered almond seeds obtained a protective effect for the hepatic tissue but no obvious protection to the testicular tissue (H & E x 200).

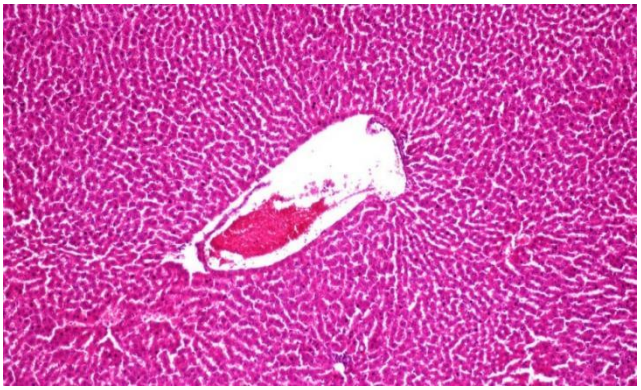

Figure 5. Rat liver poisoned with CCL4 given 15% powdered almond seeds showing mild hepatocellular deterioration and scattered necrosis; hepatic parenchymal cells are rather well preserved (H and E x 200).

Conclusion

The experimental findings of the present study concluded that almond seeds are capable of exhibiting significant improving activity of immunity in hepatic rats. Also showed powdered almond seeds obtained a protective effect for the hepatic tissue.

Recommendations

- Various amounts of almond seeds are recommended to strengthen immunity in hepatic situations.
- Almond seeds at variations in concentration, most notably 10% and 15%, can recover liver tissues.

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Ethics statement: None

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