Acta Biologica Szegediensis

Volume 68(1):16-22, 2024
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs
DOI:10.14232/abs.2024.1.16-22

ARTICLE

GC-MS Characterization and evaluation of antioxidant potential and acute toxicity of *Lupinus albus* extracts

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ABSTRACT *Lupinus albus* L., a leguminous plant, plays a therapeutic role due to its richness in bioactive compounds, which have diverse applications in human health, as a green fertilizer, and as fodder. This study aims to characterize *Lupinus albus* extracts using Gas Chromatography-Mass Spectrometry (GC-MS) and determine their total phenolic content (TPC) and total flavonoid content (TFC). The antioxidant activity (via DPPH free radical scavenging assay) and acute toxicity in Wistar rats were also evaluated. Results showed that the ethanolic extract exhibited the highest TPC (59.66 ± 0.56 mg GAE/g), while the methanolic extract had the highest TFC (14.44 ± 0.20 mg QE/g). GC-MS analysis identified 20 compounds in the ethanolic extract, with the major constituents being lupanine (54.34%), valeric acid (22.26%), and isolinoleic acid (20.94%). The ethanolic extract demonstrated significant antioxidant activity, with an IC50 of 0.86 ± 0.01 mg/mL. Acute toxicity tests indicated that the *L. albus* extract contributed to decreased cholesterol and stabilized glycemia at a dose of 2000 mg/kg body weight.
Acta Biol Szeged 68(1):16-22 (2024)

KEY WORDS
antioxidant activity
Lupinus albus
polyphenols
toxicity

ARTICLE INFORMATION
Submitted
13 April 2024
Accepted
30 December 2024
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Introduction

Medicinal plants are valuable resources due to their richness in bioactive compounds, which serve as extranutritional constituents and represent a diverse range of molecules (Ben Younes et al. 2018). Numerous studies have demonstrated the beneficial effects of these compounds in combating various diseases (Hartman et al. 2018).

Excessive reactive oxygen species (ROS) can induce oxidative stress, resulting in cellular damage and contributing to serious illnesses such as diabetes mellitus, hypertension, obesity, and cardiovascular diseases (Dontha 2016). Antioxidant phytochemicals have gained importance as a defense against chronic diseases caused by oxidative stress (Siger 2012).

Among the most well-known natural remedies in the Tiaret region of Algeria is *Lupinus albus* L., traditionally used to treat diseases such as diabetes and hypercholesterolemia. This species belongs to the genus *Lupinus* and the Fabaceae family. It is cultivated for human consumption, as green manure, and as fodder due to its richness in carbohydrates, proteins, and fibers (Elsawi et al. 2018). The bioactive components of lupin have been utilized therapeutically for thousands of years, benefiting conditions such as diabetes, hypertension, obesity, cardiovascular diseases, and colorectal cancer (Prusinski 2017).

This study aims to biologically evaluate *L. albus* extracts through GC-MS characterization, determination of total phenolic and flavonoid contents, evaluation of antioxidant activity, and acute toxicity testing in Wistar rats.

Materials and Methods

Plant material and extraction

The seeds of *Lupinus albus* L. were procured from a local herbalist in Tiaret, Algeria (35° 23′ 17″ N, 1° 19′ 22″ E). The seeds were cleaned, air-dried, and ground into powder. Extracts were prepared by maceration (Ghezelbash et al. 2015) using a ratio of 5 g of powder to 50 mL of ethanol, methanol (80:20 v/v), or distilled water. The mixtures were stirred continuously at room temperature for 24 h, filtered, and evaporated at 40 °C to obtain the extracts.

Determination of total phenolic content

Table 1. Extraction yields (%) of *Lupinus albus* extracts

Extract	Methanolic	Ethanolic	Aqueous
Yield (%)	40 ± 8.88	43.66 ± 3.78	26 ± 0.00

Results are expressed as mean ± SEM, n = 3

The total phenolic content was measured using the Folin-Ciocalteu method (Blainski et al. 2013). A 0.2 mL sample of each extract (1/128 to 1 mg/mL dilutions) was mixed with 1 mL of freshly prepared 10% Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate (Na_2CO_3). After 30 min of incubation at room temperature, the absorbance was measured at 765 nm. Results were expressed as mg gallic acid equivalent (GAE) per gram of dry plant material using a gallic acid calibration curve.

Determination of total flavonoid content

The flavonoid content was determined using the aluminum chloride method (Dehpour et al. 2009). A 0.5 mL sample of each extract was mixed with 1.5 mL of 95% methanol, 100 µL of 10% aluminum chloride ($AlCl_3$), 100 µL of 1M sodium acetate, and 2.8 mL of distilled water. After 30 min of incubation in the dark, the absorbance was measured at 415 nm. Results were expressed as mg quercetin equivalent (QE) per gram of dry plant material using a quercetin calibration curve.

GC-MS characterization

Bioactive compounds in the ethanolic extract of *L. albus* were characterized using GC-MS (7890A, Agilent Technologies) equipped with an HP-5 MS capillary column (30 m × 0.25 µm × 250 µm) and an inert mass selective detector (5975C, Agilent Technologies). Helium gas was used as the carrier, with an ionization energy of 70 eV and a flow rate of 1 mL/min. The MS transfer temperature was set to 250 °C, and the column temperature was programmed from 60 °C (initial) to 200 °C at 5 °C/min, held isothermal for 5 min, and finally raised to 250 °C at 10 °C/min. Samples (1 µL, 1:100 dilution in acetone) were injected in split mode (50:1), and compound identification was based on retention times.

DPPH free radical scavenging activity

Antioxidant activity was assessed using the DPPH assay (Braca et al. 2002). Equal volumes of extract dilutions (0.009 to 5 mg/mL) and 0.004% DPPH methanolic solution were incubated at room temperature for 30 min. Absorbance was measured at 517 nm, and the percentage inhibition was calculated using the equation:

$$\% \text{ Inhibition} = [(Ac - Ae) / Ac] \times 100.$$

where Ac is the absorbance of the control solution, and Ae is the absorbance of the extract.

Acute toxicity

The acute oral toxicity of the ethanolic extract was assessed in Wistar rats following OECD guidelines (423) (Raju and Reddy 2017). Eight male Wistar rats (268.17 ± 14.18 g) were divided into two groups (n = 4): Group 1 (negative control) received distilled water, and Group 2 received 2000 mg/kg ethanolic extract via gavage. Observations were recorded at intervals (30 min, 1, 2, 3, and 24 h) and daily for 14 days. After 15 days, rats were sacrificed, blood samples were collected for biochemical analysis, and organs were examined for macroscopic toxicity lesions.

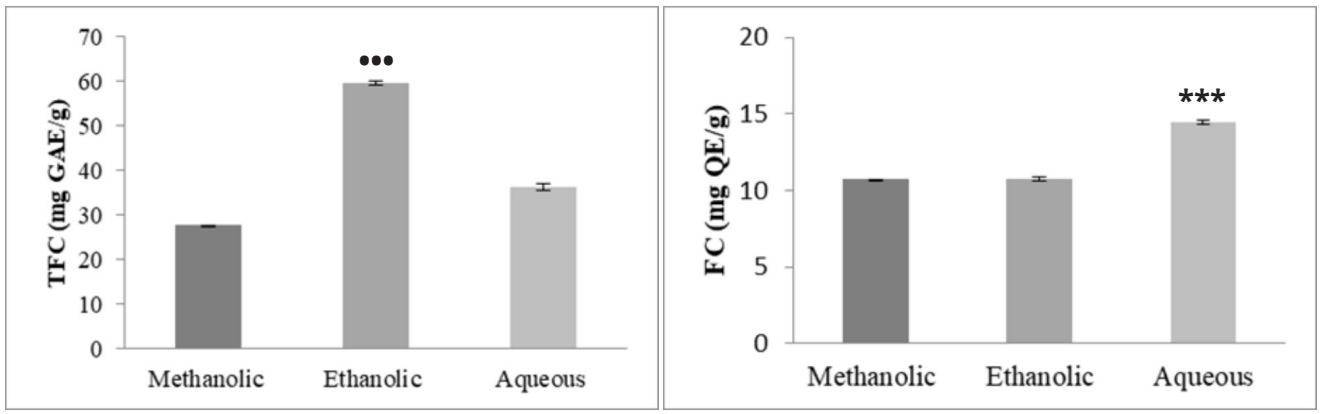

Figure 1. Total phenolic content (TPC) and flavonoid content (TFC) in *L. albus* extracts. Results are expressed as mean ± SEM (n = 3). Asterisks (***) indicate a highly significant difference between the aqueous extract and the methanolic and ethanolic extracts ($p < 0.001$). Circles (●●●) indicate a highly significant difference between the ethanolic extract and the methanolic and aqueous extracts ($p < 0.001$).

Statistical analysis

Results are presented as mean ± SEM and analyzed using STATISTICA software (version 8.0.725.0). Significance was set at $P<0.05$.

Results and discussion

Extraction yields

The extraction yields of *L. albus* showed no significant differences between the ethanolic (43.66 ± 3.78%), methanolic (40 ± 8.88%), and aqueous extracts (26%) (Table 1). These results were superior to those reported by Buszewski et al. (2019), where the yield of 96% ethanolic extract of *L. luteus* was 3.25%. The higher yields observed for the ethanolic (80%) and methanolic (80%) extracts compared to the aqueous extract can be attributed to the polarity of the solvents. The combination of organic solvents with water enhances the extraction of soluble compounds (Safdar et al. 2016). Additionally, the extraction method and solvent concentration played a role; Safdar et al. (2016) noted that the maceration technique outperformed ultrasound extraction, with 80% ethanolic extract providing the best yield due to its solvent polarity.

Total phenolic content

The ethanolic extract of *L. albus* exhibited significantly higher total phenolic content (TPC) (59.66 ± 0.56 mg GAE/g) compared to the methanolic (27.68 ± 0.09 mg GAE/g) and aqueous extracts (36.23 ± 1.16 mg GAE/g) ($p \leq 0.001$) (Fig. 1). These findings align with those of Karamać et al. (2018), who observed similar TPC rates in methanolic extracts of *L. albus* grains, including different varieties such as Vladimir, Ilmen, Bozkir, and Dedegin. However, our results contrast with the values reported by Dalaram (2017) for other *Lupinus* species, including *L. angustifolius* and *L. luteus*, which had TPC values below 7 mg GAE/g. The variations in TPC among studies might stem from differences in *Lupinus* species, extraction methods, and solvent types. Lampart-Szczapa et al. (2003) also highlighted that hydro-ethanolic extracts of *L. albus* were richer in phenolic compounds than hydro-methanolic or hydro-acetone extracts.

Total flavonoid content

The total flavonoid content (TFC) of the aqueous extract (14.44 ± 0.20 mg QE/g) was significantly higher than that of the methanolic (10.73 ± 0.04 mg QE/g) and ethanolic extracts (10.74 ± 0.22 mg QE/g) ($p \leq 0.001$) (Figure 1). These results were lower than those reported by Hanania et al. (2018, 2019), who found higher flavonoid content in 80% ethanolic extracts of *L. albus* grains, depending on whether the grains were soaked or unsoaked before extraction. Other studies, such as Al Juhaimi et al. (2019), also confirmed the richness of *L. albus* grains in flavonoids (13 mg EC/g). Variations in TFC may be influenced by genetic and environmental factors, solvent solubility, and diffusion rates (Abarca-Vargas et al., 2019).

Table 2. GC-MS profile of *L. albus* ethanolic extract. The chemical composition is presented as retention time (RT) in minutes, percentage area (%), and identified compounds

N°	RT (min)	Area (%)	Chemical compound
1	2.305	22.26	Valeric acid
2	2.400	20.94	Isolinoleic acid
3	2.727	54.34	Lupanine
4	4.589	0.19	Piperazine
5	6.853	0.26	Hexanal
6	8.788	0.16	3-Buten-2-amine
7	9.757	0.12	Heptanal
8	12.546	0.06	4-Ethylcyclohexanol
9	12.906	0.08	DL-Leucine
10	13.730	0.07	D-Limonene
11	13.842	0.13	2-Oxabicyclo[2.2.2]octane
12	14.616	0.12	Butanamine
13	16.012	0.25	Nonanal
14	16.132	0.10	Thujone
15	17.329	0.11	(+)-2-Bornanone
16	20.559	0.13	Arginine
17	22.060	0.11	2,4-Decadienal
18	23.291	0.12	Butanamine
19	42.986	0.05	Methylpent-4-enylamine
20	47.299	0.16	Propanamide

GC-MS analysis

GC-MS analysis of the ethanolic extract of *L. albus* identified 20 compounds, with the major constituents being lupanine (54.34%), valeric acid (22.26%), and isolinoleic acid (20.94%) (Fig. 2, Table 2). These findings agree with Elawadi et al. (2018), who also identified lupanine and valeric acid as key compounds in *L. albus* extracts. Additionally, studies by Mazumder et al. (2020) and Ben Hassine et al. (2021) corroborate the presence of lupanine as a major compound in *Lupinus* species. Lupanine and its derivatives, known as quinolizidine alkaloids, are associated with antioxidant, anticholinergic, and antidiabetic activities, including glucose homeostasis and insulin secretion stimulation (Wiedemann et al. 2015). Valeric acid exhibits neuroprotective and anti-anxiety effects (Gharib et al. 2015; Vishwakarma et al. 2016), while isolinoleic acid demonstrates antioxidant, anti-inflammatory, and hypocholesterolemic properties (Rahman et al. 2014).

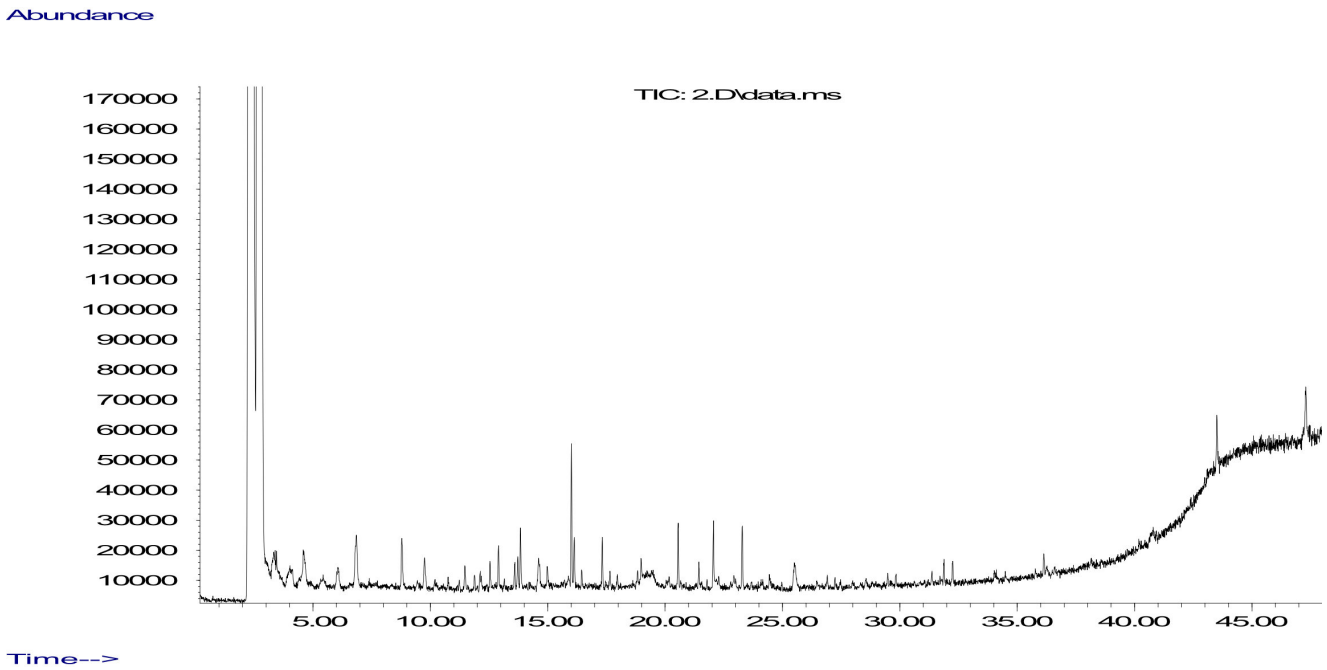

Figure 2. Chromatogram of the GC-MS analysis of the ethanolic extract of L. albus.

Antioxidant activity

The antioxidant activity, measured as the minimum concentration required to inhibit 50% of DPPH radicals (IC50), showed a significant difference between ethanolic (0.86 ± 0.01 mg/mL) and methanolic (4.34 ± 0.19 mg/mL) extracts, while no significant difference was observed between ethanolic and aqueous extracts (1.67 ± 0.04 mg/mL) (Table 3). These findings align with Buszewski et al. (2019), who reported a DPPH inhibition rate of 83.6% for 70% ethanolic extracts of *L. luteus*. Variability in antioxidant activity among solvents highlights the superior extraction efficiency of ethanol for antioxidant compounds. Zhou and Yu (2004) emphasized the influence of solvent type on the extraction of antioxidants and free radical inhibition capacity. A positive correlation between phenolic content and antioxidant activity has also been observed (Tosun et al. 2009).

Table 3. IC_{50} values of methanolic, ethanolic, and aqueous extracts of *L. albus*

Extract	$IC5_0$ (mg/mL)
Methanolic	4.34± 0.19
Ethanolic	0.86 ± 0.01***
Aqueous	1.67 ± 0.04***

The IC_{50} values represent the concentration (mg/mL) required to inhibit 50% of DPPH radicals. Results are expressed as mean ± SEM (n = 3). Asterisks indicate significant differences compared to the methanolic extract.

Evolution of body weight

The results of weight change showed no significant difference between the group receiving ethanolic extract of *L. albus* (GTEL) versus the negative control group (GT-) with a value of 288.06 ± 17.85 g and 255.12 ± 5.48 g, respectively. In parallel, a food intake of 255.25 ± 21.68 g/week and 250.125 ± 1.59 g/week was consumed by the GTEL and GT- group, respectively (Fig. 3). In fact, the biochemical blood tests showed no significant difference (Fig. 4) in blood glucose between GT- and GTEL (111 ± 8 and 89 ± 3.6 mg/dl, respectively). In contrast, the cholesterol results showed a highly significant decrease in the GTEL group (28.25 ± 9 mg/dl) compared to the GT- group (61 ± 11 mg/ml). No significant difference for urea, creatinine, triglycerides was reported between the two groups (66.5 ± 4.3; 11.17 ± 5.62 and 88.5 ± 3 mg/dl) vs (43± 3.7; 10.34± 9.27 and 79.75± 2 mg/dl) for the GTEL and GT- group, respectively. A significant increase in TGO and TGP in the GTEL group (200.07 ± 57.23 and 90.25 ± 19.9 IU/l) vs. the GT- group (100.18 ± 37.86 and 50.28 ± 11.51 IU/l). Results indicated a decrease in cholesterol for the GTEL group. This decrease can be explained by the hypocholesterolemic effect of lupin proteins. This result was confirmed by Prusinski et al. (2017). These proved that the consumption of lupin caused a reduction in cholesterol. In 2004, Sirtori proved that lupin proteins played a major role in the reduction of cholesterol based

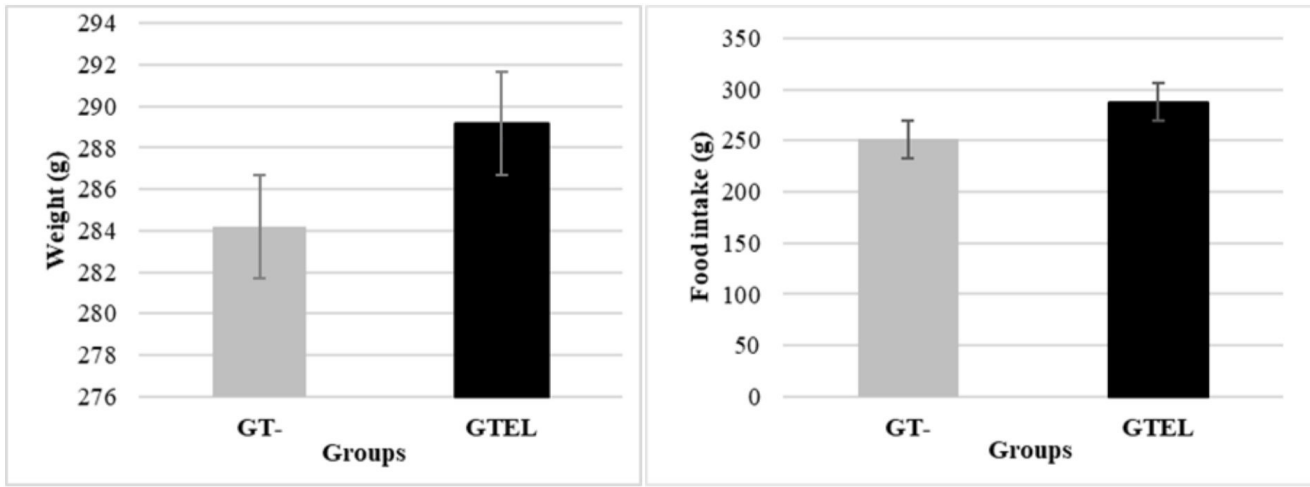

Figure 3. Weight and food intake of Wistar rats during the experimental period. Results are expressed as mean ± SEM (n = 4). Changes in body weight and food intake were monitored to evaluate the effects of the extracts.

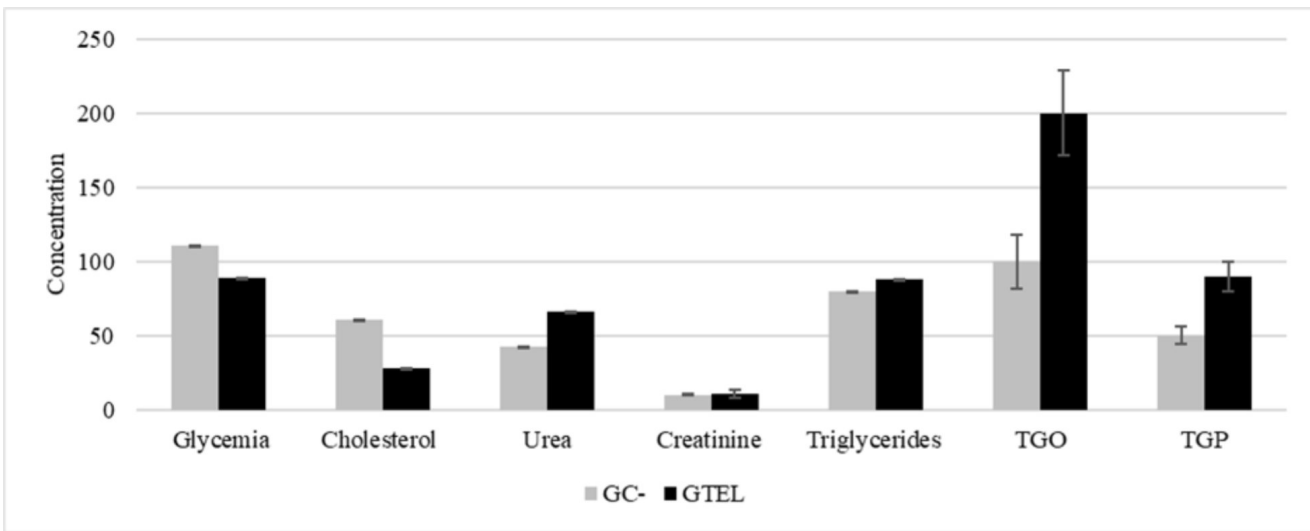

Figure 4. Biochemical blood parameters of Wistar rats after treatment with *Lupinus albus* extracts. Blood glucose, urea, cholesterol, and triglycerides are expressed in mg/dL; creatinine is expressed in mg/L; TGO (AST) and TGP (ALT) are expressed in IU/L. Results are presented as mean ± SEM (n = 4). Asterisks (**) indicate a highly significant difference between the GTEL group and the GT- group ($p < 0.001$).

on the activity of LDL receptors of human hepatoma cell lines. Other work showed that cholesterol reduction was related to metabolic processes (Fontanari et al. 2012). In addition, the modulation of decrease of intestinal cholesterol absorption was done by the bay of bile acids of which the consumption of blue lupin promoted the metabolism of bile acids as regulators of several metabolic processes (Martins et al. 2005; Staels et al. 2009). In parallel, our results indicated an increase in urea. This aggravation was judged by the high protein ratio of lupin. These results were in line with those of Gang-Jee et al. (2020) where the high-protein diet caused an increase in urea due to damage to the function and structure of the kidneys. Panasiewicz et al. (2022) showed that *Lupinus* grains were considered a very important source of high-quality proteins. Concerning the increase of liver enzymes (TGO and TGP) in the GTEL group may be related to liver function against consumption of new molecules (Mirmiran et al. 2019; Paramastri et al. 2021). TGO and TGP tests are markers of liver impairment. Research indicated that the progression of liver enzymes related to the expression of excessive fat deposition in the liver (Amany et al. 2013).

Acknowledgements

Authors would like to thank the engineers of Laboratory Reproduction of Farm Animals and Laboratory of Animal Hygiene and Pathology of Institute of Veterinary Sciences University Ibn Khaldoun Tiaret. Thanks also to the Directorate General of Scientific Research and Technological Development "DGRSDT".

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