Physicochemical Properties of Oat Cookies Supplemented with Avocado and its Effect on Immune Deficiency in Rats

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العنوان: كلية التربية النوعية . جامعة المنيا . جمهورية مصر العربية

الخصائص الفيزيائية والكيميائية لكوكيز الشوفان المضاف إليها الأفوكادو وتأثيره على نقص المناعة في الفئران

فاكهة الأفوكادو تُعد واحدة من أكثر البدائل الواعدة كبديل للزبدة بفضل ارتفاع قيمتها الغذائية. تناولت هذه الدراسة تأثير كوكيز الشوفان المُدعّم بالأفوكادو كبديل للزبدة على الجرذان التي تعاني من نقص المناعة، بالإضافة إلى تقييم التركيب الكيميائي للكوكيز وخصائصه الحسية ونشاطه المضاد للأكسدة. تم اضافة الأفوكادو لكوكيزالشوفان بنسبة 50%، 75%، و100%. أظهرت التقييمات الحسية أن الكوكيز المُعد بنسبة 100% من الأفوكادو كان الأكثر تقبلاً بشكل عام. وكشفت التحليلات الكيميائية أن محتوى البروتين والألياف والرماد والرطوبة زادت بزيادة نسبة الأفوكادو، بينما انخفض محتوى الكربوهيدرات والدهون والسعرات الحرارية. كما أدى إضافة الأفوكادو إلى كوكيز الشوفان إلى زيادة مستويات مضادات الأكسدة، والبوليفينولات الكلية، والفلافونويدات الكلية. حيث تم استخدام 18 جرذاً أبيضاً ذكراً وزن (170 ± 10 جم) وقسمت إلى مجموعتين. المجموعة الأولى (n=6) الضابطة السالبة بينما تم حقن المجموعة الثانية (n=12) بسيكلوسبورين بجرعة 50 مجم/كجم/يوم تحت الجلد في زيت الزيتون لمدة 10 أيام لإحداث نقص المناعة. بعد تأكيد الإصابة بنقص المناعة، تم تقسيم المجموعة الثانية إلى مجموعتين فرعيتين: الاولى تغذت على كوكيز الشوفان (الضابطة الموجبة)، والثانية تغذت على كوكيز الشوفان المُدعّم بالأفوكادو بنسبة 100%. بعد 8 أسابيع، أظهرت الجرذان التي تغذت كوكيز الشوفان المُدعّم بالأفوكادو بنسبة 100%100% تحسنًا كبيرًا في مستويات الجلوبولين المناعي مقارنة بالمجموعة الضابطة الموجبة حيث ارتفعت مستويات IgG ، IgM، و IgA إلى 4.31، 3.12، و0.92 نانوجرام/مل، على التوالي، مقارنةً بـ 2.23، 1.67، و0.53 نانوجرام/مل في المجموعة الضابطة الموجبة بالإضافة إلى ذلك، أظهرت مجموعة OC+A 100% تحسنًا في نسبة الدهون، وظائف الكلى والكبد، وكذلك في صورة الدم.علاوة على ذلك، ارتفعت الأنشطة المضادة للأكسدة حيث سجلت مستويات إنزيمي سوبر أكسيد ديسميوتاز و الكاتاليز (13.91, 15.54 U/L).بينما انخفضت مستويات MDA إلى 88.32 نانومول/لتر. في المقابل، أظهرت المجموعة الضابطة الموجبة مستويات أقل من إنزيمي سوبر أكسيد ديسميوتاز و الكاتاليز ومستويات أعلى من MDA دعمت نتائج الفحص النسيجي لأنسجة الطحال الدراسات البيوكيميائية. تشير الدراسة إلى أن إضافة الأفوكادو يعزز القيمة الغذائية لكوكيز الشوفان ويلعب دورًا وقائيًا في تحسين نقص المناعة والإجهاد التأكسدي في الفئران.

الكلمات المفتاحية: الخصائص الفيزيائية والكيميائية، الشوفان، البسكويت، الأفوكادو، نقص المناعة.

Physicochemical Properties of Oat Cookies Supplemented with Avocado and its Effect on Immune Deficiency in Rats

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Abstract

Avocado fruit (*Persia americana*) is one of the most promising alternatives as a fat substitute because of its unique nutritional composition. This study investigated the effects of oat cookies supplemented with avocado as a butter substitute on immune-deficient rats and evaluated the cookies' chemical composition, sensory properties, and antioxidant activity. It is being incorporated into oat cookies at 50%, 75%, and 100% levels. The sensory evaluation revealed that cookies with 100% avocado had the overall acceptance. The chemical composition revealed that protein, fiber, ash, and moisture contents increased with the increased ratio of avocado puree, while the carbohydrate content, fat content, and calories decreased. The addition of avocado puree to oat cookies increased the levels of antioxidant parameters, including DPPH%, total phenols, and total flavonoids. In the biological study, eighteen male albino rats (170 ± 10 g) were divided into two groups. Group I (n=6) served as the negative control, while **Group II** (n=12) received Cyclosporine at a dosage of 50 mg/kg/day, subcutaneously in olive oil for 10 days to induce immune deficiency. After confirmation of immune suppression, Group II was divided into two subgroups: one fed on oat cookies (positive control) and the second fed on oat cookies supplemented with 100% avocado. After 8 weeks, rats fed on OC+A100% showed significant improvement in immunoglobulin parameters compared to the control (+ve) group, with IgG, IgM, and IgA levels increasing to 4.31, 3.12, and 0.92 ng/mL, respectively, compared to 2.23, 1.67, and 0.53 ng/mL in the control (+ve). Additionally, showed improvement in lipid profile, kidney, liver functions and hematological in serum. Moreover, the antioxidant activity elevated in OC+A 100% group, SOD and CAT enzyme (13.91 U/L and 15.54 U/L). While MDA decreased to 88.32 nmole/L. In contrast, the control (+ve) group showed lower SOD, CAT and higher MDA levels. Our biochemical studies corroborated the histology of spleen. These results suggest that avocado supplementation enhances the nutritional value of oat cookies and has a protective role in ameliorating immune deficiency and oxidative stress in rats.

Key Words: Physicochemical, Oat, Cookies, Avocado, Immune Deficiency.

Introduction

The immune system is a sophisticated network of organs, cells, and proteins that safeguards the body from infections while preserving its own cells. The immune process relies heavily on accurate cell-cell communication for optimal operation, and any disruption to the signaling systems involved may lead to diminished immune system reactivity. Sufficient levels of antioxidants are necessary to prevent damage to immune cells **(Adhikari and Tirosh, 2012)**. Antioxidant substances naturally inhibit hydrolytic and oxidative digestion enzymes, exhibit anti-inflammatory properties, and perform many biological or therapeutic functions, in addition to scavenging damaging reactive oxygen species **(Sachdeva *et al*., 2014)**.

The dietary status of an individual significantly impacts the efficacy of the immune system. Undernutrition, resulting from insufficient intake of micronutrients, may hinder the body's capacity to facilitate innate immunological responses **(Cai et al., 2020)**. Fruits, vegetables, and grains are excellent sources of natural antioxidants, comprising diverse antioxidant components **(Ravimannan and Nisansala, 2017)**.

Cookies are highly popular in the human diet, mostly due to their palatable flavor, convenience, affordability, and extended shelf life. Consequently, they serve as superior snack options and possible vehicles for health-enhancing components **(Sozer *et al*., 2014)**. The advancement of snack foods characterized by low glycemic index, elevated dietary fiber content, and qualities that enhance satiety has garnered significant scientific attention **(Zhang *et al*., 2021)**.

Oat (*Avena sativa*) has garnered significant interest for its unique multifunctional qualities, attributed to its high dietary fiber content and the phytochemicals found in the grain **(Rasane *et al*., 2015)**. Oats are abundant in vital amino acids, unsaturated fatty acids, vitamins, minerals, and antioxidants. Besides its nutritious features, certain research indicates that the majority of individuals with celiac disease can tolerate oats **(Othman *et al*., 2011)**. Due to the use of high fat content in cookies, substituting them with fruits, vegetables, or cereals results in healthier alternatives **(Hayek and Ibrahim, 2013)**. The development of alternative cookies can incorporate traditional medicinal plants, such as avocado **(Brahmachari, 2011)**.

Avocado (*Persea americana*) is a fruit from an evergreen tree indigenous to Mexico, Central America, and South America, but it is now

cultivated globally **(Talabi *et al*., 2016)**. Rich in antioxidants, vitamins, monounsaturated fatty acids, fiber, and potassium. The avocado fruit holds immense value as a food source, medicinal agent, and provider of high-quality oil. It is widely recognized as a functional food due to its health-enhancing phytochemicals, including glutathione and beta-sitosterol, making it suitable for making value-added food items that can enhance nutritional benefits **(Kim and Uhl, 2011)**. Nutritionally, avocado is calorie-dense and distinguished by its high unsaturated fatty acid content, along with an abundance of both lipo-soluble and water-soluble vitamins, particularly vitamins A and C **(Dreher and Davenport, 2013)**. Whereas the body produces antioxidant enzymes, supplemental dietary antioxidants are essential for enhancing immunity and protecting against the harmful effects of free radicals and oxidative stress. The aim of the study was formulating oat cookies supplemented with avocado (fat substitute) and their effect on immune deficiency in rats; the physicochemical properties was also considered.

Materials

Samples

Oat (*Avena sativa*), Avocado (*Persea Americana*) and all the ingredients for preparing cookies were purchased from local markets (Carrefour) in Alexandria, Egypt.

Chemicals and Kits

The chemicals and reagents utilized in this study were sourced from EL-Goumhorya Chemical Company, Egypt. Cyclosporine (CsA) was obtained from Morgan Chemical Factory in Cairo, Egypt, while the kits for biochemical parameter determination were purchased from Sigma Chemical Company, Egypt.

Animals

Normal male albino rats of the Sprague Dawley strain (n = 18), weighing about 170 ± 10 g, were obtained from the animal house of the Institute of Graduate Studies and Research, Alexandria University.

Methods

Preparation of oat cookies supplemented with avocado

Oat cookies were prepared with varying proportions of butter and avocado puree as a fat substitute, compared to a control group consisting of oat cookies (**Table 1, Figure 1, and Photo 1**). Oat flour was sifted into a clean bowl, and then mashed avocado pulp (or butter), honey, salt, milk, baking powder, and vanilla were added and blended to achieve a creamy consistency. The mixture was then thoroughly combined to form a homogeneous dough. The dough was kneaded manually on a clean, smooth surface for approximately 5 minutes. It was then rolled out on a wooden board using a rolling pin to a uniform thickness of about 4 mm. Using a cookie cutter, the dough was cut into round shapes. The cut dough pieces were arranged in a greased baking tray and baked in an electric oven at 200 °C for 20 minutes. Following baking, the cookie samples were allowed to cool immediately and were then packed in polyethylene bags, sealed, and stored in airtight containers for subsequent analysis. **(Bornare and Khan, 2015).**

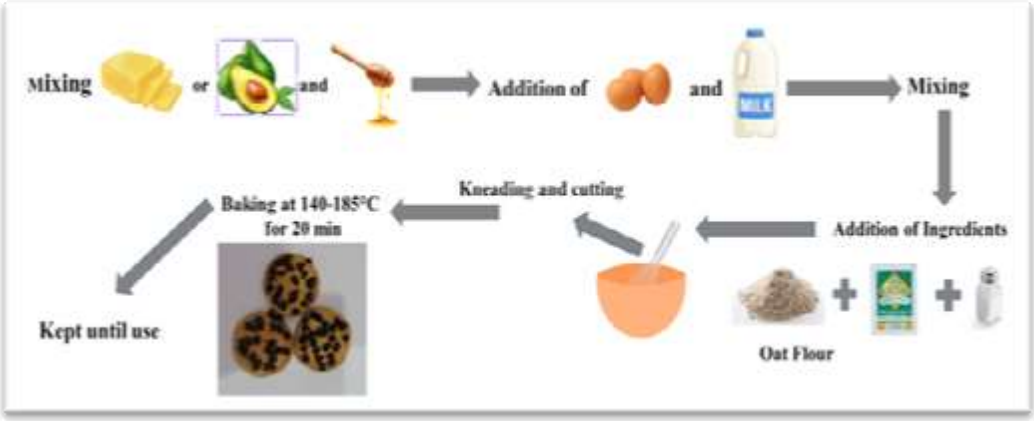

Figure 1. Cookie production process flowchart

Table (1): Ingredients used for preparation of oat cookies supplemented with avocado (fat substitute)

Ingredients	Control O-cookies	OC+A 50% Cookies (1)	OC+A 75% Cookies (2)	OC+A 100% Cookies (3)
Oat flour g	150	150	150	150
Honey g	30	30	30	30
Butter g	110	55	27.5	0.0

Avocado g	0.0	55	82.5	110
Backing powder g	5	5	5	5
Vanilla g	3	3	3	3
Salt g	0.5	0.5	0.5	0.5
Milk Tbsp*	4	2	2	2

*The amount of milk was reduced because avocado, being a fruit, has a higher water content compared to butter.

Control = Oat cookies (100% Butter), O+A- cookies (1) = Oat cookies)50% Butter and 50% avocado puree (;O+A- cookies (2) = Oat cookies (25% Butter and 75% avocado puree), O+A-cookies (3) = Oat cookies (100% avocado puree)

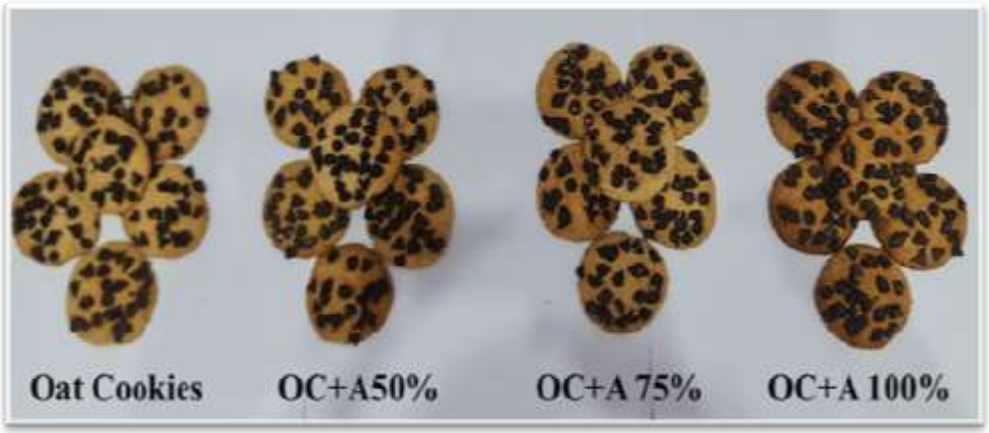

Photo 1. Preparation of Oat Cookies (OC) Supplemented with Avocado (A)

Chemical composition

The chemical composition of protein, fat, fiber, ash, and moisture content in oat cookies and oat cookies supplemented with avocado was determined using the methods established by the Association of Official Analytical Chemists **(AOAC, 2012).** Carbohydrate content was calculated as the difference, as described by the American Association of Cereal Chemists **(Radovanović *et al*,. 2010).** The calorie value per 100 g was calculated using the following formula: (9 x fat%) + (4 x protein%) + (4 x carbohydrates%) Cal

Antioxidant activities, total phenolic and flavonoids of cookies

The DPPH radical scavenging activity (antioxidant activity) was assessed using the stable radical 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), following the methodology outlined by **(Hwang and Do Thi, 2014)**.

The phenolic content in the extract was determined using both the Folin-Ciocalteu (FC) and aluminum chloride colorimetric assays, as described by **Debnath *et al*, (2017)**. For the determination of phenolic content, an aliquot of the extract (10 mg/mL) was prepared and combined with gallic acid standards ranging from 0 to 500 µg/mL. These solutions were mixed with FC reagent and incubated with sodium carbonate solution. Absorbance was measured at 700 nm, and the results were expressed as gallic acid equivalents (GAE) per 100 g of dry weight.

Total flavonoid content was assessed using catechin standards (0–25 µg/mL). A sample solution (10 mg/mL) was prepared and mixed with sodium nitrite and aluminum chloride solutions. Absorbance was measured at 517 nm, and he results were reported as catechin equivalents (CE) per 100 g of dry weight **(Wronkowska *et al*,. 2010).**

Sensory evaluation

Sensory evaluation was conducted with forty panelists, consisting of staff and students from the Faculty of Specific Education at Alexandria University. Samples of oat cookies and oat cookies supplemented with avocado were assessed for color, taste, odor, texture, crispness, and overall acceptance, following the methodology outlined by **Cross (1978)**.

Biological study

Experimental animal design:

A total of 18 male albino rats, each weighing approximately 170 ± 10 g, underwent a two-week adaptation period during which they were fed a basal diet as per the guidelines outlined by **Reeves *et al.*, (1993)**, with water provided ad libitum. Following the adaptation period, the rats were divided into two main groups. **Group I** (n=6) was fed a standard diet and served as the negative control group (-ve), while **Group II** (n=12) received Cyclosporine (CsA) at a dosage of 50 mg/kg/day, administered subcutaneously in olive oil for 10 days **(Suke *et al*., 2006)**. Subsequently, their health condition was monitored, and daily changes were recorded until symptoms began to appear, including lethargy, reduced movement, and lack of appetite. Then random blood samples were drawn to confirm the occurrence of immune deficiency. After that the rats of second **Group II** divided into 2 sub-groups (each 6 rats) as follows**:**

SupGroup II: (n=6 rats) were fed on a basal diet + 30% Oat Cookies and they were kept as a positive control group (+ve).

SupGroup IV: (n=6 rats) were fed on a basal diet + 30%Oat Cookies (%30)supplemented with Avocado 100% (fat substitute).

At the end of experimental period (8 weeks) rats were fasted for 12 hours. Blood were collected and centrifuged (3000r.p.m.), serum was separated and stored frozen at -20◦C for analysis.

Biochemical analysis

Immunoglobulins (IgG, IgA, and IgM) are crucial components of the immune system, playing significant roles in the body's defense against infections. They were quantified using enzyme-linked immunosorbent assay (ELISA), as described by **Burlingame and Rubin (1990)**. The total cholesterol level was determined following the methodology outlined by **Richmond (1973)**, while triglyceride (T.G.) concentrations were measured according to **Fossati and Prencipe (1982)**. High-density lipoprotein (HDL-c) levels were assessed using the method developed by **Lopes-Virella *et al.* (1977)**, and low-density lipoprotein (LDL-c) was also evaluated. Liver enzyme activities, including alkaline phosphatase (ALP) activity, were measured according to **Lavie *et al.* (2018)**, while aspartate transaminase (AST) activity was assessed following the protocol by **Yagi *et al.* (1985)**. Alanine transaminase (ALT) activity was determined using the method described by **Williamson (1974)**. Urea levels were evaluated according to the method established by **(Wuepper *et al.*, 2003)**, and creatinine concentrations were measured using the technique outlined by **Shlipak *et al.* (2013)**. The antioxidant enzyme activities of superoxide dismutase (SOD) and catalase (CAT) were assessed using the methods of **Sinha (1972) and Fridovich (1972)**, respectively. Malondialdehyde (MDA) levels were quantified based on the method developed by **Aslanturk *et al.* (2011)**. Complete blood count (CBC) parameters, including hemoglobin concentration, packed cell volume (PCV), and the counts of red blood cells (RBC) and white blood cells (WBC), were performed as per **Shawky (2015)** and **Jopling *et al.* (2009)**, with RBC and WBC counts conducted under low-power microscopy.

Histological examination:

Spleen samples were preserved in 10% neutral buffered formalin for microscopic inspection and then embedded in paraffin. Sections were

prepared at a thickness of 5 µm and then stained with Mayer's hematoxylin and eosin. The stained slices were analyzed using a light microscope at 10× magnification. The slides were photographed and recorded in accordance with the protocols established by **Banchroft *et al*. (1996)**.

Statistical analysis

Data analysis was conducted using IBM SPSS software version 23.0. Quantitative data was described using mean and standard deviation. Statistical significance was determined at the 5% level. To compare more than two groups, the F-test (ANOVA) was employed, followed by the Post Hoc test (LSD) for pairwise comparisons, as described by **Kirkpatrick and Feeney (2013)**.

Results and Discussion

Proximate chemical composition of cookies

The different parameters of chemical composition of oat cookies (OC) and oat cookies supplemented with avocado (OC+A 50%, OC+A 75%, and OC+A 100%) are shown in **Table 2**. The results revealed that the addition of avocado puree to oat cookies at ratios of 50, 75, and 100% increased the protein concentration, fiber, and moisture contents compared to OC (control). The highest protein concentration was observed in OC+A 100% with a concentration of 12.9%, followed by OC+A 75% and OC+A 50% with concentrations of 12.49% and 11.88%, respectively, compared to 11.22% for the control. However, no significant differences have been observed between the protein content in OC+A50%, OC+A 75%, and control. Similarly, fiber and ash contents increased with increasing the ratio of avocado puree added to the OC to record about 2.9 and 1.78% for OC+A 100% compared to 1.6 and 1.67% in control. However, no significant differences in ash content have been observed between all treatments. Conversely, fat and carbohydrate contents decreased with increasing the ratio of avocado puree added to reach about 15.14% and 49.89% for OC+A 100% compared to 20.29% and 60.68%, respectively, for control. while the carbohydrates content in the three groups supplemented with avocado puree was significantly different from control. Additionally, the obtained calories in the three groups supplemented with avocado puree were significantly lower than that of the control 470.21 with the OC+A 100% being the least in calories obtained with a value of 387.42 Cal. The findings align with previous studies that have reported the use of avocado as a supplementary ingredient in various products. **Adie *et al*., (2020)** reported the nutritional

properties of produced cookies by the addition of avocado pulp instead of butter at ratios of 20, 30, 40, and 50%. The moisture, ash, and fiber contents increased with increased avocado ratio to record 14.6, 2.09, and 0.62% at 50% ratio compared to 11.13, 1.4, and 0.29%, respectively, for the control sample (100% butter). Also, the same study noted that fat and carbohydrate contents decreased when the ratio of avocado pulp increased to record 16.0 and 59.7% compared to 18.03 and 61.31%, respectively, for control. Conversely, the protein content decreased with an increasing proportion of avocado pulp, which is inconsistent with our findings. Additionally, corn chips supplemented with avocado paste at 10% increased the contents of ash and fiber and decreased the carbohydrate contents, while the moisture content decreased **(Zuñiga-Martínez *et al*., 2024)**. **Eteng *et al*., (2020)** clarified that producing cake supplemented with avocado puree at 20, 40, 80, and 100% instead of butter increased the protein, fiber, ash, and moisture contents. These parameters were 16.85, 2.10, 2.52, and 11.65% for 100% wheat/avocado cake compared to 12.33, 1.44, 2.10, and 6.99%, respectively, for 100% wheat/butter (control). Also, the fat content decreased to reach 20.67% for 100% wheat/avocado cake compared to 29.51% for control.

Table (2): Chemical composition of oat cookies and oat cookies supplemented with avocado (fat substitute)

Variables / Treatment groups	Protein	Fat	Fiber	Ash	Carbohydrates	Moisture	Calorie
	(g/100g)						CaL
(Oat cookies)	11.22 ^{a}±0.40	20.29 ^{a} ±0.17	1.6 ^{b} ±0.09	1.67 ^{a} ±0.13	60.68 ^{a} ±0.39	4.54 ^{c} ±0.65	470.21^{a}±0.60
OC+A50%	11.88 ^{ab}±0.24	18.80 ^{ab} ±0.39	2.1 ^{b} ±0.22	1.71 ^{a} ±0.24	52.36 ^{b} ±0.43	13.15 ^{b} ±0.46	426.16 ^{b}±0.36
OC+A75%	12.49 ^{ab}±0.61	16.88 ^{bc} ±0.59	2.5 ^{a} ±0.11	1.76 ^{a} ±0.14	49.49 ^{b} ±1.44	16.88 ^{a} ±1.30	399.84 ^{c} ±1.8
OC+A100%	12.9 ^{b} ±0.59	15.14 ^{c} ±1.05	2.9 ^{a} ±0.23	1.78 ^{a} ±0.09	49.89 ^{b} ±1.31	17.39 ^{a} ±1.42	387.42 ^{c}±1.63
F	2.295	12.353*	10.314*	0.100	26.183*	32.400*	23.936*
P	0.155	0.002*	0.004*	0.958	<0.001*	<0.001*	<0.001*
LSD 5%	1.578	2.083	0.565	0.511	3.319	3.404	24.412

Data was expressed using Mean ± SEM
F: F for ANOVA test, pairwise comparison bet. each 2 groups were done using Post Hoc Test (LSD)
Means in the same column with common letters are not significant (i.e. Means with Different letters are significant)
*: Statistically significant at $P \leq 0.05$
Control = Oat cookies (100% Butter)
OC+A50% = Oat cookies (50% Butter and 50% avocado puree)
OC+A75 = Oat cookies (25% Butter and 75% avocado puree),
OC+A 100% = Oat cookies (100% avocado puree)

Antioxidant activities, total phenolic and total flavonoids of cookies

Antioxidant activities, total phenols, and total flavonoids **Table 3**. shows that all parameters increased when the ratio of avocado puree was increased. The OC+A50%, OC+A 75%, and OC+A 100% were significantly higher in DPPH scavenging activity (%) than control with values of 62.23, 80.31, and 39.91%, respectively. Similarly, total phenol and flavonoid compounds were significantly higher than control in OC+A 75% and OC+A 100% with values of 223.4 and 295.21 mg GAE/100g for total phenols and 2.88 and 3.7 mg CE/100g for total flavonoids.

The obtained results are comparable to **Zuñiga-Martínez *et al*., (2024),** who concluded that total phenols, total flavonoids, and DPPH% in corn chips supplemented with avocado increased with increasing the ratio of avocado added (2-10%). **Arueya *et al*.,(2021)** reported that total phenols, total flavonoids, and DPPH% in a snack of maize flour increased when the ratio of avocado seed flour added to the snack increased. Total phenols, total flavonoids, and DPPH% recorded 392.0 mg/100 g, 146.23 mg/100 g, and 54.09% at 30% of avocado compared to 252.74 mg/100 g, 138.14 mg/100 g, and 39.0% for control (100% maize flour).

Table (3): Antioxidant activities, total phenolic and total flavonoids of oat cookies and oat cookies supplemented with avocado (fat substitute)

Parameters / Treatment groups	DPPH scavenging activity (%)	Total phenols (mg GAE /100g)	Total flavonoids (mg CE /100g)
Control (Oat cookies)	39.91 ^{c} ±1.55	140.2 ^{c} ±17.41	1.30 ^{c} ±0.15
OC+A50%	41.15 ^{c} ±1.06	146.6 ^{c} ±18.1	1.92 ^{c} ±0.19
OC+A75%	62.23 ^{b} ±1.54	223.40 ^{b} ±15.9	2.88 ^{b} ±0.17
OC+A100%	80.31 ^{a} ±4.19	295.21 ^{a} ±16.2	3.7 ^{a} ±0.36
F	63.146*	18.679*	20.915*
P	<0.001*	0.001*	<0.001*
LSD 5%	7.891	55.162	0.754

Data was expressed using Mean ± SEM
F: F for ANOVA test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test (LSD)
Means in the same column with common letters are not significant (i.e. Means with Different letters are significant)
*: Statistically significant at $P \leq 0.05$
Control = Oat cookies (100% Butter)
OC+A50% = Oat cookies (50% Butter and 50% avocado puree)
OC+A75 = Oat cookies (25% Butter and 75% avocado puree),
OC+A 100% = Oat cookies (100% avocado puree)
GAE, gallic acid equivalents; CE, catechin equivalents.

Sensory evaluation of cookies

The parameters of sensory characteristics including color, taste, odor, texture, crispness, and general acceptance of oat cookies (OC) and oat cookies supplemented with avocado (OC+A 50%, OC+A 75%, and OC+A 100%) treatments are shown in **Table 4**. The data indicated non-significant differences between all treatments in sensory characters with increased ratios of avocado except color and texture. The acceptance scores for OC+A at 50% (7.65), 75% (7.65), and 100% (7.53) showed no significant differences compared to the control group (7.94). In contrast, the color and texture scores differed significantly between the avocado-supplemented groups and the control. Specifically, the color and texture scores were as follows: OC+A 50% (7.02 and 7.63), OC+A 75% (6.97 and 7.48), and OC+A 100% (6.08 and 7.14), respectively. According to sensory evaluation, we chose 100% avocado puree which has no impact on the sensory qualities of the cookies. This makes avocado a suitable fat substitute, offering health benefits such as antioxidants and healthy fats, while maintaining consumer acceptability. Thus, this ratio is also, selected for use in the rat experiment to assess its effect on enhancing immunity. Our results agreed with **Hussein *et al*., (2021)**, who reported that odor showed a steady decline in values as the butter was substituted with avocado. In food applications, partial substitution of Butter with avocado pulp in cookie production has been successful, resulting in nutritious cookies with acceptable organoleptic qualities up to a 30% substitution level **(Wekwete and Navder, 2008)**. Additionally, avocado seed flour has been investigated as a partial substitute for wheat flour in cookies, with a 50% substitution rate yielding the best results in terms of taste, color, texture, and aroma **(Hussein *et al*., 2021)**. In contrast, **Eteng *et al*., (2020)** noted that color, taste, aroma, texture, and general acceptance of wheat cakes decreased significantly with the increased ratio of avocado added. However, all samples of wheat/avocado cakes were generally acceptable to the panelists. Also, color, texture, taste, aroma, and general acceptance of cookies supplemented with 20, 30, 40, and 50% of avocado decreased with increasing the ratio of avocado **(Zuñiga-Martínez *et al*., 2024)**. Moreover, cookies of wheat flour supplemented with 10% of avocado peel showed a decrease in taste, crispness, aroma, appearance, and general acceptance with values of 7.2, 6.6, 6.4, 6.1, and 6.7 compared to 7.6, 7.1, 7.4, 7.3, and 7.6 for cookies of 100% wheat flour **(Olaoye *et al*., 2019)**. The decrease in color and taste acceptability of cookies supplemented with avocado could be attributed to the dull color of avocado and the presence of

tannins, as has been documented by **Eteng *et al.* (2020)** in wheat/avocado cakes.

Table (4): Sensory evaluation of oat cookies and oat cookies supplemented with avocado

Variables / Treatment groups	Color	Taste	Odor	Textures	Crispness	Acceptance
Control (oat cookies)	7.52^{a} ±0.19	8.32^{a} ±0.12	9.00^{a} ±0.10	8.18^{a} ± 0.23	8.85^{a} ±0.22	7.94^{a} ±0.09
OC+A50%	7.02^{b} ±0.21	8.19^{a} ±0.11	8.79^{a} ±0.40	7.63^{b} ±0.12	8.61^{a} ±0.18	7.65^{a} ±0.11
OC+A75%	6.97^{b} ±0.09	7.97^{a} ±0.06	8.12^{a} ±0.63	7.48^{b} ±0.14	8.38^{a} ±0.11	7.56^{a} ±0.10
OC+A100%	6.08^{c} ±0.06	7.88^{a} ±0.41	8.07^{a} ±0.59	7.14^{b} ±0.15	8.24^{a} ±0.21	7.53^{a} ±0.28
F	15.718*	0.794	0.968	6.828*	0.575	1.290
P	0.001*	0.531	0.454	0.013*	0.647	0.342
LSD 5%	0.493	0.732	0.679	0.541	1.150	0.606

Data was expressed using Mean ± SEM
F: F for ANOVA test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test (LSD)
Means in the same column with common letters are not significant (i.e. Means with Different letters are significant)
*: Statistically significant at $P \leq 0.05$
Control = Oat cookies (100% Butter)
OC+A50% = Oat cookies (50% Butter and 50% avocado puree);
OC+A75 = Oat cookies (25% Butter and 75% avocado puree),
OC+A 100% = Oat cookies (100% avocado puree)

Effect of oat cookies and oat cookies supplemented with avocado (100%) on the lipid profile of the rats with induced immune deficiency

The parameters of lipid profile in rats with induced immune deficiency fed on OC and OC+A 100% are displayed in **Table 5**. The results revealed that rats in the control (+ve) group exhibited significantly high levels in the parameters of lipid profile, including cholesterol, triglyceride (T.G) and low-density lipoprotein (LDL) with values of 136.3, 160.6, and 72.54 mg/dl, respectively. While, it was significantly less in high-density lipoprotein (HDL), with a value of 16.5 compared to 35.3 and 31.3 for OC and OC+A 100%, respectively. The feeding of rats on OC+A 100% decreased the levels of cholesterol (72.21 mg/dl), T.G (75.8 mg/dl), and LDL (25.12 mg/dl), and increased the level of HDL (31.3 mg/dl), and their levels were not significantly different from those of the control (-ve) group.

These results are in accordance with **Miñón-Hernández *et al.* (2021)** who reported that the ingestion of avocado peel by rats consuming a high-sucrose and fate diet attenuated the high levels of cholesterol and T.G and

enhanced the lower levels of HDL. Also, a mixture of 1:1 avocado seeds and garlic aqueous extract modulated the lipid profile in rats with metabolic syndrome induced by a high-calorie diet by lowering the levels of cholesterol and T.G and enhancing the levels of HDL **(Azantsa *et al*., 2022).** Moreover, **Muzakar and Fudholi (2019)** proved that avocado reduced the levels of T.G and LDL by up to 20% and 22%, respectively. The pretreatment of rats intoxicated with CCl_4 with 200 mg of aqueous extract of avocado reduced the levels of total cholesterol (46.57 mg/dl) and triacylglycerol (46.28 mg/dl) compared to 70.79 and 126.94 mg/dl for rats in the CCl_4-intoxicated group **(Brai *et al*., 2020).** Further, **Nasef and Ahmed (2019)** noted that there was a significant reduction in the levels of total cholesterol, T.G and LDL and an elevation in the level of HDL in hypocholesterolemic rats when they were treated with avocado fruit powder compared to the control(+ve).The monounsaturated fatty acids (MUFAs) in avocado may primarily account for its positive effects on blood lipids by lowering LDL and plasma cholesterol levels without increasing triglycerides **(Colquhoun *et al*., 1992; Pieterse *et al.*, 2005; Meyer et al., 2011).**

Table (5): Effect of oat cookies and oat cookies supplemented with avocado (fat substitute) on lipid profile in immune-deficient rats

Treatment groups	Cholesterol (mg/dl)	T.G (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control(-)	69.21 ^{b} ±5.33	73.1 ^{b} ±4.71	35.3 ^{a} ±1.95	19.87 ^{b} ±1.60
control (+)	136.3 ^{a} ±16.41	160.6 ^{a} ±16.92	16.5 ^{b} ±1.44	72.54 ^{a} ±5.64
OC+A100%	75.21 ^{b} ±5.57	75.8 ^{b} ±4.24	31.3 ^{a} ±1.62	25.12 ^{b} ±1.31
F	12.565*	22.762*	34.708*	69.996*
P	0.007*	0.002*	0.001*	<0.001*
LSD 5%	36.242	36.091	5.817	12.0

Data was expressed using Mean ± SEM
F: F for ANOVA test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test (LSD)
Means in the same column with common letters are not significant (i.e. Means with Different letters are significant)
*: Statistically significant at $P \leq 0.05$
OC+A 100% = Oat cookies (100% avocado puree)

Effect of oat cookies and oat cookies supplemented with avocado100% on the immunoglobulin parameters of rats with induced immune deficiency

The immunoglobulin parameters of rats with induced immune deficiency treated with OC and OC+A 100% are displayed in **Table 6**. The rats in the control group(+ve) showed a significant decrease in all parameters, including IgG, IgM, and IgA, with values of 2.23, 1.67, and 0.53 ng/mL, respectively, compared to rats in the control(-ve) and OC+A 100% groups. However, the treatment of these rats with OC+A 100% increased the levels of IgG (4.31 ng/mL), IgM (3.12 ng/mL), and IgA (0.92 ng/mL). The current results agree with **Saad (2020),** who indicated that the cyclosporine-administered rats showed a significant decrease in IgA, IgM, and IgG with values of 0.34, 0.34, and 0.55 ng/µL, respectively, compared to the control(-ve). However, the levels of these parameters were increased significantly when these rats were treated with avocado seed extract to reach 0.52, 0.66, and 0.92 ng/µL, respectively. In addition, there was a significant increase in the levels of IgM and IgG in rats with induced immune deficiency when treated with avocado fruit and seed extracts at a ratio of 5 and 10% **(Negm and Abo-Raya, 2018).** The enhancement of immune functions may be related to the chemical composition of avocado fruit that contains high levels of antioxidant compounds such as phenols, flavonoids, alkaloids, saponins, steroids tannins, phytosterols and vitamins (E, ascorbic acid, and Carotene). **Cuevas *et al*., 2015.**

Table (6): Effect of oat cookies and oat cookies supplemented with avocado (fat substitute) on immunoglobulin in immune-deficient rats

Treatment groups	IgG(ng/ml)	IgM (ng/ml)	IgA (ng/ml)
Control(-)	6.01 ^{a} ±0.40	4.36 ^{a} ±0.25	0.950 ^{a} ±0.11
control (+)	2.23 ^{c} ±0.15	1.67 ^{c} ±0.16	0.53 ^{b} ±0.09
OC+A100%	4.31 ^{b} ±0.27	3.12 ^{b} ±0.19	0.92 ^{a} ±0.10
F	128.080*	133.930*	14.375*
P	<0.001*	<0.001*	0.005*
LSD 5%	0.579	0.403	0.314

Data was expressed using Mean ± SEM
F: F for ANOVA test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test (LSD)
Means in the same column with common letters are not significant (i.e. Means with Different letters are significant)
*: Statistically significant at P ≤ 0.05
OC+A 100% = Oat cookies (100% avocado puree)

Effect of oat cookies and oat cookies supplemented with avocado % 100 (fat substitute) on liver enzymes and kidney function in immune-deficient rats

The rats in the control (+ve) group displayed the highest activity of liver enzymes, including alanine Aminotransferase (ALT) at 51.5 U/L, aspartate Aminotransferase (AST) at 155.1 U/L, and alkaline Phosphatase (ALP) at 70.2 U/L which varied significantly from that of the control(-ve) and OC+A 100% **(Table 7)**. However, these rats, when fed on OC+A 100%, retrieved the activity of these enzymes to record values of 26.21, 55.2, and 35.31 U/L, respectively. In addition, no significant differences have been observed in liver enzymes between rats in the control(-ve) and OC+A 100% groups except for ALT activity. For kidney functions, the levels of creatinine and urea in rats in the control (+ve) group were significantly higher than those of the control (-ve) and OC+A 100%groups, with values of 1.8 and 56.0 mg/dl, respectively. Also, the feeding of rats on OC+A 100% decreased the levels of creatinine (0.84 mg/dl) and urea (32.1 mg/dl), which did not vary significantly from those of the control.

The current results clearly indicated that feeding rats on OC+A 100% retrieved the liver enzyme activity to about their normal activity. The same trend was observed in a previous study of Azantsa ***et al.*** **(2022)** in which the formulation 1:1 of avocado/garlic aqueous extract restored the activity of liver enzymes in rats with induced metabolic syndrome by lowering their activity compared to the rats in the control(+ve). While, **Okpala *et al.* (2022)** concluded that rats with calcium carbide induced hepatotoxicity treated with avocado seed extract showed no significant differences in activity of ALT, ALP, and AST from that of the control (-ve) (rats fed water and feed). However **El-Kholie *et al.* (2023)** demonstrated that treatment with a 5% avocado-apricot mixture reduced the levels of ALT (93.97 U/L), AST (75.72 U/L), and ALP (73.29 U/L) in infected with immunocompromised rats to 39.75, 41.67, and 36.22 U/L, respectively. Additionally, the feeding of rats with induced immune deficiency on OC+A 100% reduced the levels of creatinine, and urea to reach about the normal levels. These findings agree with **El-Kholie *et al.* (2023),** who mentioned that the treated hepatic rats with a 5% avocado/apricot mixture returned the renal biomarkers such as urea and creatinine to approximately their normal values. Also, 7 days of dosing albino rats with avocado retrieved renal biomarkers and serum creatinine to an acceptable level in rats treated with meloxicam **(Anshar *et al.,* 2018)**. According to **Elmoslemany *et al.* (2021)**, rats treated with cyclosporine demonstrated significantly elevated levels of

uric acid (6.56 mg/dl), urea (74.23 mg/dl), and creatinine (1.46 mg/dl). Following treatment with avocado seed powder, these levels showed a marked reduction, with uric acid decreasing to 3.62 mg/dl, urea to 32.35 mg/dl, and creatinine to 0.93 mg/dl.

Table (7): Effect of oat cookies and oat cookies supplemented with avocado (fat substitute) on liver and kidney enzymes in immune-deficient rats

Parameters / Treatment groups	ALT U/L	AST U/L	ALP U/L	Creatinine Mg/dl	Urea Mg/dl
Control(-)	20.1 ^{c} ±1.37	50.21 ^{b} ±1.86	31.3 ^{b} ±1.42	0.71 ^{b} ±0.16	27.3 ^{b} ±1.65
control (+)	51.2 ^{a} ±1.83	155.1 ^{a} ±13.1	70.2 ^{a} ±4.73	1.8 ^{a} ±0.14	56.0 ^{a} ±2.30
OC+A100%	26.21 ^{b} ±1.34	55.2 ^{b}±4.60	35.31 ^{b}±2.86	0.84 ^{b} ±0.17	32.1 ^{b} ±2.12
F	116.094*	53.740*	42.227*	14.903*	56.668*
P	<0.001*	<0.001*	<0.001*	0.005*	<0.001*
LSD 5%	5.292	27.932	11.394	0.534	7.067

Data was expressed using Mean ± SEM
F: F for ANOVA test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test (LSD)
Means in the same column with common letters are not significant (i.e. Means with Different letters are significant)
*: Statistically significant at $P \leq 0.05$
OC+A 100% = Oat cookies (100% avocado puree)

Effect of oat cookies and oat cookies supplemented with avocado (fat substitute) on antioxidant enzymes in rats with induced immune deficiency

Table (8), presents the levels of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA), in immune-deficient rats treated with oat cookies supplemented with 100% avocado (OC+A 100%). All enzyme activity was significantly higher in the rats of OC+A 100% group than that of the control (+ve) with values of 13.91 U/L and 15.54 U/L for SOD and CAT. While the level of MDA decreased (88.32 nmole/L,). The treatment of rats with control (+ve) decreased the SOD, CAT, and MDA activities with values of 7.43 U/L, 8.80 U/L, and 110.09 nmole/L, respectively. All enzyme activity in rats fed on O-100% did not vary significantly from that of control(-ve).

The obtained results are incompatible with **Elmoslemany *et al.* (2021),** who indicated that treated rats with cyclosporine exhibited a significant increase in the levels of MDA and a decrease in the levels of SOD and CAT

compared to the control(-ve). However, the treatment of these rats with avocado seed powder ameliorated their levels by decreasing the levels of MDA and increasing the levels of SOD and CAT. Also, the rats with hepatocarcinogenesis showed a marked decrease in the levels of GST, SOD, and GSH, but their treatment with avocado extract modulated their levels by increasing (Glutathione S-transferase (GST), SOD, and Glutathione (GSH) activities to be roughly at their normal levels **(Ahmed *et al*., 2022)**. **Radwan *et al.* (2022)** showed that treatment of rats with monosodium glutamate-induced nephrotoxicity with avocado fruit extract retrieved antioxidant enzymes by enhancing the activities of SOD and CAT. The use of avocado as an antioxidant agent could be attributed to the presence of phytochemicals including phytosterols, triterpenes, fatty acids and phenolic compounds **(Wang *et al.*, 2010; Mushtaq and Wani, 2013; Padilla-Camberos *et al.*, 2013)**.

Table (8): Effect oat cookies and of oat cookies supplemented with avocado (fat substitute) on superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) in immune-deficient rats

Treatment groups	SOD (U/ L)	CAT (U/ L)	MDA((nmol/L)
Control(-)	9.21 ^{b} ±0.92	9.82 ^{b} ±0.61	79.2 ^{b} ±3.49
Control (+)	7.43 ^{b} ±0.74	8.80 ^{b} ±0.95	110.09 ^{a} ±6.74
OC+A100%	13.91 ^{a} ±1.46	15.54 ^{a} ±1.76	88.32 ^{b} ±5.30
F	56.668*	9.060*	8.810*
P	<0.001*	0.015*	0.016*
LSD 5%	3.751	4.177	18.504

Data was expressed using Mean ± SEM
F: F for ANOVA test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test (LSD)
Means in the same column with common letters are not significant (i.e. Means with Different letters are significant)
*: Statistically significant at $P \leq 0.05$
OC+A 100% = Oat cookies (100% avocado puree)

Effect of oat cookies and oat cookies supplemented with avocado (fat substitute) on red and white blood cell in immune-deficient rats

The hematological parameters of rats with induced immune deficiency fed on OC+A100% are shown in **Table 9**. The rats in the control(+ve) group showed a significant decrease in red blood cells (RBCs) with a value of $2.58x10^{6}$ /mm^{3} compared to $5.57x10^{6}$ /mm^{3} and 4.31 x 10^{6} /mm^{3} for rats in the control (-ve) and OC+A100% groups. In contrast, the rats in the control

(+ve) group showed the highest level of) white blood cells (WBCs) with a value of $9.21x10^3$ /mm^3, which varied significantly from that of the control(-ve) ($5.56x10^3$ /mm^3) and OC+A100% ($6.26x10^3$/mm^3). The hemoglobin concentration in the rats of the control (+ve) group was significantly decreased with a value of 8.07 g/dl compared to 14.54 and 12.16 g/dl for the rats in the control (-ve) and OC+A1 00% groups, respectively. In a similar way, the packed cell volume (PCV%) values in the rats of the (-ve) control (56.13%) and OC+A100% (52.12%) groups were significantly higher than that of the (+ve) control group (31.2%).

These findings are in accordance with **Brai *et al.* (2020),** who mentioned that the treatment of CCl_4-intoxicated rats with aqueous leaf extract of avocado ameliorated the hematological parameters by increasing the PCV% and hemoglobin from 33.5% and 11.17 g/dl in CCl_4-inoxicated rats to 38.67% and 12.89 g/dl for intoxicated rats treated with avocado extract. **Saad (2020)** reported that rats treated with cyclosporine showed a significant decrease in RBCs, hemoglobin, and WBCs. The levels of R.B. Cs ($2.76x10^6$ /mL), hemoglobin (9.35 g/dl), and WBCs ($1.63x10^3$/µm) in cyclosporine-administered rats were increased significantly to $3.86x10^6$ mL, 11.25 g/dl, and $4.6x10^3$/µm , respectively, when they were treated with avocado seed extract.

Table (9): Effect of oat cookies and oat cookies supplemented with avocado (fat substitute) on red and white blood cell in immune-deficient rats

Parameters / Treatment groups	R.B.Cs (10^6/mm3)	W.B.Cs (10^3/mm3)	Hemoglobin g/dl	PCV %
Control(-)	5.57^a±0.15	5.56^b±1.13	14.54^a±0.16	56.13^a ±0.63
control (+)	2.58^b±0.10	9.21^a±1.20	8.07^b±0.11	31.2^b ±3.41
OC+A100%	4.31^a±0.14	6.26^b±1.10	12.16^a±0.13	52.12^a ±5.66
F	16.744*	19.039*	16.832*	110.160*
P	0.004*	0.003*	0.003*	<0.001*
LSD 5%	1.270	1.536	2.761	7.687

Data was expressed using Mean ± SEM
F: F for ANOVA test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test (LSD)
Means in the same column with common letters are not significant (i.e. Means with Different letters are significant)
*: Statistically significant at $P \leq 0.05$
OC+A 100% = Oat cookies (100% avocado puree)

Histological examinations of spleen tissue

A photomicrograph of spleen tissue from the control (-) group revealed normal histological structure, showing red pulp (RP) and splenic sinusoids (S) with splenic cords, as well as well-defined white pulp (WP) comprising lymphoid follicles, including the marginal zone (MZ), mantle zone (M), germinal center (GC), and central arteriole (→), as illustrated in Figure (2A). In contrast, the spleen tissue from the control (+ve) group exhibited significant degenerative changes in the white pulp (WP), characterized by dense, irregular nuclei and vacuolated cytoplasm (thick arrow). Additionally, congestion (thin arrow), dilation, hemorrhage (star), and heightened activation of the red pulp (RP) with a loss of normal sinusoidal architecture were observed, as shown in Figure (2B). However, the spleen tissue from rats treated with the OC+A100% group showed near-normal architecture, with red pulp (RP) and white pulp (WP) structures largely retained. Mild sinusoid dilation (S) and minimal hemosiderin deposition (→) were also noted, as demonstrated in Figure (2C). These results suggest the potential protective effect of the OAC-100% treatment on spleen structure, possibly due to its anti-inflammatory and antioxidant properties. The spleen from rats fed on OC+A100%group showed significant recovery in its structure compared to the control positive group. The spleen retained near-normal architecture, with reduced damage to the white pulp and decreased hemosiderin deposition. This improvement in immune response may be attributed to the avocado in the diet, which is rich in antioxidants like vitamin E and carotenoids. These compounds are known to enhance immune function by reducing oxidative stress and inflammation.

Previous studies have demonstrated the immune-boosting and protective effects of avocado, which align with the findings of this study. In the current study, splenic sections from the control positive group showed diffuse, ill-defined white pulp and congested blood vessels in the red pulp, consistent with the results reported by **Khattab *et al.* (2019)**, who observed that Cyclosporine A (CsA) caused significant reductions in white pulp cellularity and congestion in the red pulp. Conversely, the group that received avocado-seed-supplemented cookies showed improved spleen architecture, supporting **Saad (2020)** findings that avocado seeds can enhance immune function by increasing serum immunoglobulins and antioxidant enzyme activity, partially mitigating CsA-induced spleen damage. Furthermore, **Arukwe *et al.* (2012)** indicated that phenolic

compounds in avocado act as anti-inflammatory agents, antioxidants, and immune enhancers, which aligns with the improvements seen in the spleen's structure in my study. **Thacker and Janssen (2012)** emphasized the spleen's critical role in immune response regulation, particularly in the activation of T and B cells and antibody production, further supporting the observed enhancements in splenic function.

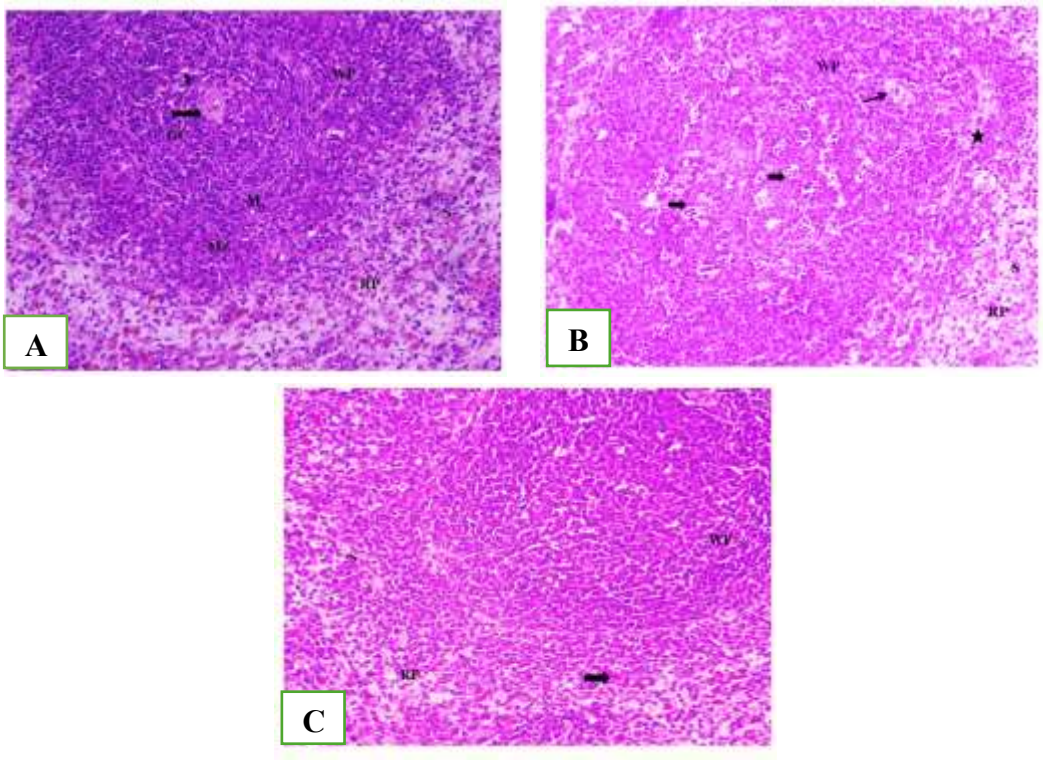

Figure (2): A photomicrograph of spleen treated groups **A:** Control (-), **B:** Control (+), **C:** OC+A100% (**H&E x 40**).

Conclusion

The study concluded that supplementing oat cookies with avocado as a fat substitute significantly improved their nutritional profile by reducing fat and carbohydrate contents while increasing protein, fiber, and antioxidant activity. Sensory evaluation showed that cookies with 100% avocado were well accepted, making them a suitable fat alternative. The consumption of oat cookies supplemented with 100% avocado improved lipid profiles, liver enzyme activities, kidney function, and antioxidant enzyme levels in immune-deficient rats. Additionally, the supplementation increased immunoglobulin parameters, with higher levels of IgG, IgM, and IgA

observed in rats fed the avocado-supplemented cookies. Histological examination of spleen tissue confirmed these findings, showing near-normal architecture in treated rats. These results support the use of avocado as a healthy fat substitute in functional foods to enhance immune function and overall health.

Ethical Approval

All study experiments were ethically approved by the Scientific Research Ethics Committee from the University of Alexandria, Animal Ethics Committee, Faculty of Medicine (**Approval no2023-8-13 SREC 0306301**).

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References

Adhikari, K. B., & Tirosh, O. (2012). Alcoholic Liver Disease and Immune Response. *The Open Gastroenterology Journal, 6*, 8-15.

Adie, P., Enenche, D., Atsen, T., & Ikese, C. (2020). Physicochemical and sensory evaluation of cookies produced by partial substitution of margarine with Avocado (Persia americana) Pulp. *Asian Food Science Journal, 18*(2), 41-47.

Ahmed, O. M., Fahim, H. I., Mohamed, E. E., & Abdel-Moneim, A. (2022). Protective effects of Persea americana fruit and seed extracts against chemically induced liver cancer in rats by enhancing their antioxidant, anti-inflammatory, and apoptotic activities. *Environmental Science and Pollution Research, 29*(29), 43858-43873.

Anshar, A. R., Bahar, M. A., & Ikliptikawati, D. K. (2018). The effect of avocado to the profile of blood urea nitrogen (BUN) and creatinine in rats (Rattus norvegicus) induced with meloxicam. *Journal of The Indonesian Veterinary Research, 2*(1), 1-7.

A.O.A.C. 2012. International Official Methods of Analysis. 19th ed., Gaithersburg, MD: AOAC International.

Arueya, G. L., Oluwatobi, A., & Arueya, G. (2021). Avocado (Persia americana) Seed Processing into a Third Generation (3 g) Functional Food Snack: Nutritional, Antioxidative Stress and Safety Potentials. *African Journal of Food Science and Technology, 12*, 1-15.

Arukwe, U., Amadi, B., Duru, M., Agomuo, E., Adindu, E., Odika, P. . . . & Anudike, J. (2012). Chemical composition of Persea americana leaf, fruit and seed. *International Journal of Research and Reviews in Applied Sciences, 11*(2), 346-349.

Aslanturk, A., Kalender, S., Uzunhisarcikli, M., & Kalender, Y. (2011). Effects of methidathion on antioxidant enzyme activities and malondialdehyde level in midgut tissues of Lymantria dispar (Lepidoptera) larvae. *Journal of the Entomological Research Society*, *13*(3), 27-38.

Azantsa, B. K., Raissa, N. F., Mary-Ann, M. A., Amelie, M., Alexine, K., Cliffbrown, M. . . . & Laure, N. J. (2022). Lipomodulatory and anti-oxidative stress effects of a polyherbal formulation based on garlic and avocado seed extracts on high fat high sucrose diet fed rats. *Metabolism Open, 15*, 100195.

Banchroft, J., Stevens, A., & Turner, D. (1996). *Theory and practice of histological techniques* (4th ed.). London: Churchil Livingstone.

Bornare, D. T., and Khan, K. S. A. (2015). Physical and Sensory Evaluation of Cookies Incorporated with Oats and Honey. International Journal of Engineering Research and Technology, 4.

Brahmachari, G. (2011). Bio-flavonoids with promising antidiabetic potentials: A critical survey. *Research signpost, 661*(2), 187-212.

Brai, B., Falode, J., Adisa, R., & Odetola, A. (2020). Effects of aqueous leaf extract of avocado (Persea americana) on total cholesterol, triacylglycerols, protein and haematological parameters in CCl 4-intoxicated rats. *Clinical Phytoscience, 6*, 1-6.

Burlingame, R. W., & Rubin, R. L. (1990). Subnucleosome structures as substrates in enzyme-linked immunosorbent assays. *Journal of immunological methods, 134*(2), 187-199.

Cai, H., Chen, L., Yin, C., Liao, Y., Meng, X., Lu, C. . . . & Wang, X. (2020). The effect of micro-nutrients on malnutrition, immunity and therapeutic effect in patients with pulmonary tuberculosis: a systematic review and meta□analysis of randomised controlled trials. *Tuberculosis, 125*, 101994.

Colquhoun, D. M., Moores, D., Somerset, S. M., & Humphries, J. A. (1992). Comparison of the effects on lipoproteins and apolipoproteins of a diet high in monounsaturated fatty acids, enriched with avocado, and a high-carbohydrate diet. *The American journal of clinical nutrition, 56*(4), 671-677.

Cross, R. (1978). Training and testing of judges for sensory analysis of meat quality. *Food Technology, 36*, 48-54.

Cuevas, E., Camino, C., Benedetti, A., Basart, S., Terradellas, E., Baldasano, J. M., ... & Schulz, M. (2015). The MACC-II 2007–2008 reanalysis: atmospheric dust evaluation and characterization over northern Africa and the Middle East. Atmospheric chemistry and physics, 15(8), 3991-4024.

Debnath, T., Ghosh, M., Lee, Y. M., Nath, N. C. D., Lee, K.-W., & Lim, B. O. (2017). Identification of phenolic constituents and antioxidant activity of Aloe barbadensis flower extracts. *Food and Agricultural Immunology*. doi: 10.1080/09540105.2017.1358254.

Dreher, M. L., & Davenport, A. J. (2013). Hass avocado composition and potential health effects. *Critical reviews in food science and nutrition, 53*(7), 738-750.

El-Kholie, E., Serag El-Deen, M., Mohamed, N., & El-Banna, S. (2023). Protective Effects of Apricot and Avocado Kernels Powder in Carbon Tetra Chloride-Induced Liver Disorder in Rats. *Journal of Home Economics-Menofia University, 33*(04), 39-50.

Elmoslemany, A. M., El-Magd, M. A., Ghamry, H. I., Alshahrani, M. Y., Zidan, N. S., & Zedan, A. M. (2021). Avocado seeds relieve oxidative stress-dependent nephrotoxicity but enhance immunosuppression induced by cyclosporine in rats. *Antioxidants, 10*(8), 1194.

Eteng, G., Olaitan, N., Iombor, T., & Igbua, F. (2020). Physico-chemical and sensory properties of wheat-margarine-avocado cake blends. *European Journal of Nutrition and Food Safety, 12*(40), 66-74.

Fossati, P., & Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical chemistry, 28*(10), 2077-2080.

Fridovich, I. (1972). Superoxide radical and superoxide dismutase. *Accounts of Chemical Research*, *5*(10), 321-326.

Hayek, S. A., & Ibrahim, S. A. (2013). Consumer acceptability of chocolate chip cookies using applesauce as a fat (butter) substitute. *Emirates Journal of Food & Agriculture (EJFA), 25*(3), 159-168.

Hussein, J. B., Msheliza, E. A., & Kave, S. B. (2021). Physico-chemical and Sensory Properties of Cookies Produced by Partial Substitution of Margarine with Avocado Pear (Persia americana). *Croatian journal of food science and technology, 13*(1), 96-104.

Hwang, E.-S., & Do Thi, N. (2014). Effects of extraction and processing methods on antioxidant compound contents and radical scavenging activities of laver (Porphyra tenera). *Preventive nutrition and food science, 19*(1), 40.

Jopling, J., Henry, E., Wiedmeier, S. E., & Christensen, R. D. (2009). Reference ranges for hematocrit and blood hemoglobin concentration during the neonatal period: data from a multihospital health care system. *Pediatrics*, *123*(2), e333-e337.

Khattab, H. A., Abounasef, S. K., & Bakheet, H. L. (2019). The biological and hematological effects of Echinacea purpurea L. Roots extract in the immunocompromised rats with cyclosporine. *Journal of microscopy and ultrastructure, 7*(2), 65-71.

Kim, M. J., & Uhl, K. (2011). Sex and lifestyle drugs: The pursuit of the fountain of youth. *Clinical Pharmacology & Therapeutics, 89*(1), 3-9.

Kirkpatrick, L. A., & Feeney, B. C. (2013). *A simple guide to IBM SPSS statistics for version 20.0*. Belmont: Cengage Learning.

Lavie, C. J., Laddu, D., Arena, R., Ortega, F. B., Alpert, M. A., & Kushner, R. F. (2018). Reprint of: healthy weight and obesity prevention: JACC health promotion series. *Journal of the American College of Cardiology, 72*(23 Part B), 3027-3052.

Lopes-Virella, M. F., Stone, P., Ellis, S., & Colwell, J. A. (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical chemistry*, *23*(5), 882-884.

Meyer, M. D., Landahl, S., Donetti, M., & Terry, L. A. (2011). Avocado. In L. A. Terry (Ed.), *Health- Promoting Properties of Fruit& Vegetables* (p.p. 27-50). Oxford: CAB International Oxfordshire.

Miñón-Hernández, D., Dorantes-Alvarez, L., Guzmán-Gerónimo, R. I., Alvarado-Olivarez, M., Herrera-Meza, S., Santiago-Roque, I. . . . & Arrieta-Baez, D. (2021). Avocado creole peel ameliorates metabolic alterations caused by a high sucrose fat diet in a wistar rats model. *Plant Foods for Human Nutrition, 76*, 12-19.

Mushtaq, M., & Wani, S. (2013). Polyphenols and human health-A review. *International Journal of Pharma and Bio Sciences, 4*(2), B338-B360.

Muzakar, L., & Fudholi, A. (2019). Effectiveness of Zack's cocoter avocado juice against blood pressure on old age hypertension patients in Palembang. International Journal of Engineering and Advanced Technology, 8(4), 740-744.

Nasef, A. N., & Ahmed, A. (2019). Effect of Bioactive Compounds of Avocado (Persea Americana) Fruit Powder on Hypercholesterolemic Rats: Biological and Biochemical Studies. *Alexandria Science Exchange Journal, 40*(July-September), 550-559.

Negm, S. H., & Abo-Raya, A. O. (2018). Therapeutic effects of Avocado (Persea americana Mill) fruits and seeds on immune deficiency in rats. *Journal of Research in Specific Education, 4*(17), 293-301.

Okpala, N. V., Adinma, J. I., Edet, M. M., & Ezejindu, D. N. (2022). The effect of avocado seed extract (Persea Americana) on carbide induced hepatoxicity in adult Wistar rats. *International Journal of Medicine and Health Development, 27*(4), 344-349.

Olaoye, O. A., Ekeh, J. I., Okakpu, C. J., & Uka, A. C. (2019). Consumer acceptability and quality characteristics of cookies produced from composite flours of wheat and banana/avocado peels. *Annals: Food Science & Technology, 20*(1), 56-64.

Othman, R. A., Moghadasian, M. H., & Jones, P. J. (2011). Cholesterol-lowering effects of oat β-glucan. *Nutrition reviews, 69*(6), 299-309.

Padilla-Camberos, E., Martínez-Velázquez, M., Flores-Fernández, J. M., & Villanueva-Rodríguez, S. (2013). Acute toxicity and genotoxic

activity of avocado seed extract (Persea americana Mill., cv Hass). *The Scientific World Journal, 2013*(1), 245828.

Pieterse, Z., Jerling, J., Oosthuizen, W., Kruger, H., Hanekom, S., Smuts, C., & Schutte, A. (2005). Substitution of high monounsaturated fatty acid avocado for mixed dietary fats during an energy-restricted diet: effects on weight loss, serum lipids, fibrinogen, and vascular function. *Nutrition, 21*(1), 67-75.

Radovanović, B.C., A.N. Radovanović and J.M. Souquet. 2010. Phenolic profile and free radical-scavenging activity of Cabernet Sauvignon wines of different geo graphical origins from the Balkan region. J. Sci. Food Agric. 90: 2455 -2461.

Radwan, E. H., Elbeltagy, A., Ibrahim, R., Tabl, G., & Nazeh, N. (2022). Evaluation of ameliorating role of avocado Persea americana fruit extract against monosodium glutamate-induced toxicity in pregnant female albino rats and their offspring. *Journal of Bioscience and Applied Research, 8*(4), 275-294.

Rasane, P., Jha, A., Sabikhi, L., Kumar, A., & Unnikrishnan, V. (2015). Nutritional advantages of oats and opportunities for its processing as value added foods-a review. *Journal of food science and technology, 52*, 662-675.

Ravimannan, N., & Nisansala, A. (2017). Study on antioxidant activity in fruits and vegetables–A Review. *International Journal of Advanced Research in Biological Sciences, 4*(3), 93-101.

Reeves, P. G., Nielsen, F. H., & Fahey Jr, G. C. (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *The Journal of nutrition, 123*(11), 1939-1951.

Richmond, N. (1973). Colorimetric determination of total cholesterol and high density lipoprotein cholesterol (HDL-c). *Clinical Chemistry, 19*, 1350-1356.

Saad, S. (2020). The antioxidant activity of avocado seeds on immunosuppression induced by cyclosporine in rats. *Home Economic Journal, 36*(1), 93-110.

Sachdeva, M., Karan, M., Singh, T., & Dhingra, S. (2014). Oxidants and antioxidants in complementary and alternative medicine: A review. *Spatula DD, 4*(1), 1-16.

Shawky, R. M., Abd-El Khalek, H. S., & Elakhdar, S. E. (2015). Selective screening in neonates suspected to have inborn errors of metabolism. *Egyptian Journal of Medical Human Genetics*, *16*(2), 165-171.

Shlipak, M. G., Matsushita, K., Ärnlöv, J., Inker, L. A., Katz, R., Polkinghorne, K. R. . . . & Coresh, J. (2013). Cystatin C versus creatinine in determining risk based on kidney function. *New England Journal of Medicine, 369*(10), 932-943.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical biochemistry, 47*(2), 389-394.

Sozer, N., Cicerelli, L., Heiniö, R.-L., & Poutanen, K. (2014). Effect of wheat bran addition on in vitro starch digestibility, physico-mechanical and sensory properties of biscuits. *Journal of Cereal Science, 60*(1), 105-113.

Suke, S. G., Ahmed, R. S., Tripathi, A., Chakraborti, A., & Banerjee, B. (2006). Immunotoxicity of phosphamidon following subchronic exposure in albino rats. *Indian Journal of Experimental Biology, 44*, 316-320.

Talabi, J. Y., Osukoya, O. A., Ajayi, O., & Adegoke, G. (2016). Nutritional and antinutritional compositions of processed Avocado (Persea americana Mill) seeds. *Asian Journal of Plant Science and Research, 6*(2), 6-12.

Thacker, R. I., & Janssen, E. M. (2012). Cross-presentation of cell-associated antigens by mouse splenic dendritic cell populations. *Frontiers in immunology, 3*, 41.

Wang, W., Bostic, T. R., & Gu, L. (2010). Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. *Food chemistry, 122*(4), 1193-1198.

Wekwete, B., & Navder, K. P. (2008). Effects of avocado fruit puree and oatrim as fat replacers on the physical, textural and sensory

properties of oatmeal cookies. *Journal of Food Quality, 31*(2), 131-141.

Williamson, D. H. (1974). L-Alanine determination with alanine dehydrogenase. In H. U. Bergmeyer (Ed.), *Methods of enzymatic analysis* (p.p. 1679-1685). New York: Elsevier.

Wuepper, A., Tattersall, J., Kraemer, M., Wilkie, M., & Edwards, L. (2003). Determination of urea distribution volume for Kt/V assessed by conductivity monitoring. *Kidney international, 64*(6), 2262-2271.

Wronkowska, M., Zielińska, D., Szawara-Nowak, D., Troszyńska, A., & Soral-Śmietana, M. (2010). Antioxidative and reducing capacity, macroelements content and sensorial properties of buckwheat-enhanced gluten-free bread. International journal of food science & technology, 45(10), 1993-2000.

Yagi, T., Kagamiyama, H., Nozaki, M., & Soda, K. (1985). Glutamate-aspartate transaminase from microorganisms. In H. U. Bergmeyer (Ed.), *Methods in enzymology* (p.p. 83-89). New York: Elsevier.

Zhang, K., Dong, R., Hu, X., Ren, C., & Li, Y. (2021). Oat-based foods: Chemical constituents, glycemic index, and the effect of processing. *Foods*, *10*(6), 1304.

Zuñiga-Martínez, B. S., Domínguez-Avila, J. A., Robles-Sánchez, R. M., Ayala-Zavala, J. F., Viuda-Martos, M., López-Díaz, J. A. . . . & González-Aguilar, G. A. (2024). Lyophilized Avocado Paste Improves Corn Chips' Nutritional Properties and Sensory Acceptability. *Foods, 13*(8), 1220.