Therapeutic Effects of Avocado (*Persea americana Mill*) Fruits and Seeds on Immune Deficiency in Rats

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Abstract: Avocado is a widely used fruit in many countries for its nutritional and medicinal benefits. This research was conducted to investigate the effect of avocado (fruits and seeds) supplementation at different levels for 6 weeks on hematology, immune system, liver functions and serum lipid profile in rats. The methanolic extracts of fresh avocado (fruits and seeds) exposed high total phenolic (258.75 and 283.93 mg GAE/100g), flavonoid (2.89 and 3.30 mg CE/100g), antioxidant activity (89.7 and 69.5%) and ascorbic acid (9.35 and 5.22 mg), respectively. Thirty-six adult male albino rats were divided into six groups, the first group, negative control group (-ve) and fed on basal diet only, the other five groups (6 rats each) were subcutaneously injected with a single dose of sheep red blood cell (SRBC) to induced immune suppression, one group of them was served as a positive control group (+ve), while groups 3 and 4 were fed on basal diet supplemented with 5 and 10% of dried avocado fruits, respectively. Groups 5 and 6 were fed on basal diet supplemented with 5 and 10% dried avocado seeds, respectively. The results indicated that, supplementation with avocado (fruits and seeds) at 5 and 10 % to rats diets were significantly increased ($P \leq 0.05$) the mean value of IgG and IgM with 123.28, 153.28, 69.73 and 103.28%, respectively for serum IgG and 113.27, 164.60, 57.52 and 70.79%, respectively for serum IgM, compared to the positive control group. Hematological parameters were significantly increased ($P \leq 0.05$) for the groups given avocado (fruits and seeds) at different levels. Moreover, liver functions were significantly improved as well as serum albumin, compared to the positive control group, while serum lipid profiles were significantly enhanced. The present study recommended using avocado (fruits and seeds) due to stimulates the immune system of rats with immune deficiency.

Key words: Avocado, fruits, seeds, immune functions, liver functions, Hematological parameters, serum lipid profile, rats.

Introduction

Immunity process is highly based on correct cell-cell communication for ideal function, and any personal injury to the signaling systems involved may cause reduced immune system responsiveness. Adequate degrees of antioxidants will be required, as a result, to avoid destruction to the immune cells **(Adhikari and Tirosh, 2012)**. Antioxidant compounds play an natural part in inhibition of hydrolytic and oxidative digestive enzymes, anti-inflammatory action and many other biological or therapeutic activities in addition to free radical scavenging of harmful active O_2 types species

(Ignat *et al.*, 2011 and Sachdeva *et al.*, 2014). Fruits and veggies are good source of natural antioxidants which consist of various antioxidant components. As a result those are alluded to as very foods or functional foods. These anti-oxidants are carotenoids, vitamins, phenolic compounds, flavonoids, dietary glutathione and endogenous metabolites **(Ravimannan and Nisansala, 2017)**.

Healthful products from plants have obtained considerable attention in recent years for their diverse pharmacological properties، including anti-oxidants **(Takeoka and Dao, 2003)**. Avocados are a rich source of nutrients and phytochemicals, Avocado contains many alkaloids, saponins, flavonoids and steroids. It is reported that it decreases liver damage in mice **(Kawagishi *et al.*, 2001)**. It is a natural antioxidant as a result of occurrence of glutathione. The amino-acid based antioxidant glutathione is involved in immune function, lipid metabolism, detoxification, and many aspects of cellular defense and duplication **(Michel *et al.*, 2009)**. Avocado is one of the plant life that contain been widely used in medication. Avocado (*Persea Americana Mill*) belongs to the family *Lauraceae* and commonly known as crocodile pear and butter fruits. The avocado has an olive green peel and solid pale yellow pulp that is rich in essential fatty acids such as linoleic, oleic, palmitic, stearic, linolenic, capric, and myristic acids **(Brai *et al.*, 2014)**. The beta-sitosterol in avocado has an immunity against diseases such as cancers, HIV, and infection **(Comerford *et al.*, 2016)**.

Avocados are a medium energy dense fruit because about 80% of the avocado edible fruit consists of water (72%) and dietary fiber (6.8%) and has been shown to have similar effects on weight control as low- fat fruits and vegetables **(Bes-Rastrollo *et al.*, 2008 ;USDA, 2011 and Dreher and Davenport, 2013)**. Avocado peel and seeds have high contents of bioactive phytochemicals such as phenolic acids, condensed tannins, and flavonoids, including procyanidins, flavonols, hydroxybenzoic, and hydroxycinnamic acids **(Padilla-Camberos *et al.*, 2013)**. These bioactive compounds have shown various biological activities such as antioxidant and anti-inflammatory properties. The anti-inflammatory activity of phenolic compounds is largely related to their ability to scavenge oxidative radicals, which is important for cell and oxidative stress regulation **(Murakami *et al.*, 2015 and Figueroa *et al.*, 2018a,b)**.

Actually though the pulp has been most generally used all over the world, in Nigeria, the powdered seed is often combined with soups, pap and puddings in the truth that it is useful in the management of chronic hypertension **(Ozolua *et al.*, 2009)**. Avocado pulp, containing acetogenin compounds, inhibited platelet aggregation with a potential preventive impact on thrombus formation (**Rodriguez-Sanchez *et al.*, 2015)**. In addition the seed and root the avocado fruit contain antibiotic to prevent bacterial spoilage of food **(Duarte *et al.*, 2016)**. As well, avocado seed improved hypercholesterolemia, and caused the reduction and treatment of high blood pressure levels, inflammatory conditions, diabetes and boosts the immunity **(Dabas, 2013 and**

Zhao *et al.*, 2017). The present study was conducted to evaluate the therapeutic effects of avocado (*Persea americana Mill*) fruits pulp and seeds at two different levels (5 and 10%) on immune deficiency in rats.

Materials and methods

Materials

Fruit: The mature fresh avocado (*Persea americana Mill*- Family, *Lauraceae*) was purchased from local markets in Egypt, dried and taken for immediate chemical analysis.

Rats: Thirty six adult male albino rats of Sprague Dawley strain, weighing (180±5g.) were purchased from Helwan Farm for Experimental Animals, Cairo, Egypt.

Chemicals: Kits for biochemical analysis were purchased from Biodiagnostic Company for Pharmaceutical and chemicals, Dokki, Egypt. Casein, vitamins, minerals, cellulose, starch, and choline were obtained from Morgan Chemical Company, Cairo, Egypt.

Sheep red blood cells (SRBC) was obtained from VACSERA, Dokki, Egypt.

Methods

Preparation of Avocado fruit powder

The fresh avocado fruits were washed with tap water then was dried by cotton cloth to remove the excess liquid prior. The clean fruit was cut into thin slice, removing the seed, skin and any blemished portions. Then, fruit pulp was soaked in a solution prepared with added lemon juice to prevent enzymatic browning action, to drying. The pulp was dried at 40 °C by Solar Energy At the national research center and vacuum packaged until use. The seeds were chopped into small pieces. The pieces were ground into powder with a mill and were further dried in an oven set at 30°C for 3 days and then used for determination the chemical composition.

Determination of Total Phenolic Compounds

Total phenolics compounds of avocado (fruits and seeds) were determined by spectrophotometer using Folin- Ciocalteu colorimetric method according to **Asami *et al.*, (2003)**.Total phenolic content was expressed as mg of gallic acid equivalent (GAE) per 100g of sample.

Determination of Total Flavonoids

Total flavonoids content of avocado (fruits and seeds) were determined by spectrophotometer according to the method described by **Khatiwara *et al.*, (2010)**. Total flavonoid content was expressed as mg of catechin equivalent (CE) per 100 g of sample.

Determination of Ascorbic Acid

Ascorbic acid in avocado fruit and seed were analyzed by the method of **AOAC (2000)**.

Determination of Antioxidant Activity

Determination of radical DPPH scavenging activity (Antioxidant activity) was determined using the stable 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) according to **Hwang and Do Thi, (2014)**.

Identification of Individual Phenolic and Flavonoid by HPLC

Phenolic and Flavonoids compounds in avocado (fruit and seed) were determined by HPLC according to the method by **Goupy *et al.*, (1999) and Mattila *et al.*, (2000)** respectively.

Experimental animal design

Thirty-six adult male albino rats Sprague Dawely Strain weighting (180± 5 g) were housed in well aerated cages under hygienic condition and fed on basal diet for one week for adaptation. After this week the rats were divided into six groups, 6 rats each, and treated as follows: The first group (6 rats) was kept as negative control group and fed on basal diet only according to **Reeves *et al.*, (1993)**. The other five groups were subcutaneously injected with a single dose of SRBC to induced immune suppression **(Suke *et al.*, 2006)**. One group of them was served as positive control group, while groups (3, 4) of rats were fed on basal diet supplemented with (5 and 10%) avocado fruit respectively. Groups (5 and 6) of rats were fed on basal diet supplemented with (5and 10%) avocado seed respectively. At the end of the experiment (6 weeks) the rats were fasted for 12 hour, and then sacrificed under ether anesthesia. Two Blood samples were collected from medial canthus of the eyes of rats by means of fine capillary glass tubes. The first sample was collected into a tube containing disodium salt of Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant and used for assessment of erythrocytes indices. These parameters were quantified by standard hematological assay analyzer. The second blood sample was collected into a centrifuge tube without any anticoagulant and centrifuged for 15 minutes at 3000 r.p.m. to obtain serum which was stored at- 20°C until used for subsequent analysis.

Biochemical analysis

Immunoglobulin M (IgM) and immunoglobulin G (IgG) were measured according to **Ziva and Pannall, (1984)**. Total leucocytes count, red blood cell (RBC) count, haemoglobin concentrations were estimated, and packed cell volume (PCV) was determined using standard haematological technique as described by **Ochei and Kolharktar (2008)**. Serum was analyzed for the following biochemical parameter: total cholesterol by the method of **Richmond, (1973)**, HDL-cholesterol by **Albers *et al.*, (1983)**, triglyceride by **Jacobs and Vander mark, (1960)** and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by **Bergmeyer *et al.*, (1978)** and alkaline phosphatase (ALP) according to **(Roy, 1970),** while**,** albumin was estimated according to **(Weissman *et al.*, 1950)**. Calculation of LDL-c and VLDL-c by the equation of **Fridewald *et al.*, (1972)**.

Statistical analysis

The results were expressed as mean±SE. The statistical analysis was carried out by using SPSS, PC statistical software (Verion 18.0 SPSS Inc., Chieago, USA) using the Dunk 'test multiple range post-hoc test. Data were analyzed by one way analysis variance (ANOVA). The values were considered significantly different at P <0.05 **(Snedecor and Cochran, 1980)**.

Results

Total phenols, total flavonoids, antioxidant activity and ascorbic acid content of Avocado (fruit and seed) were recorded in Table (1). It contains total phenols, total flavonoids , antioxidant activity and ascorbic acid in the following concentrations (258.75 mg GAE100g, 2.89 mg CE100g, 89.7% and 9.35 mg /100g) and (283.93 mg GAE100g, 3.30 mg CE100g, 69.5% and 5.22 mg/100g) sample, respectively.

Table (1): Total phenols, total flavonoids, antioxidant activity and ascorbic acid content of Avocado (fruit and seed).

sample / Parameters	Avocado fruit pulp	Avocado seed
Total phenols (mg GAE / 100g)	258.75	283.93
Total flavonoids (mg CE/ 100g)	2.89	3.30
Ascorbic acid (mg/100g)	9.35	5.22
Antioxidant activity (DPPH, %)	89.7	69.5

GAE, Gallic acid equivalent; CE, Catchin equivalent .

Table (2): Types and concentrations (ppm) of phenolic compounds of avocado (fruits and seeds).

Phenolic compounds	Avocado fruit	Avocado seed
Syrengic	2.16	768.59
Coumarin	0.95	-
Ferulic	4.05	14.60
Salycilic	8.0	-
Cinnamic	0.15	0.35
Pyrogallol	216.37	225.73
Protocatechuic	23.24	54.22
Vanillic	3.76	50.78
Catechol	20.96	28.82
Caffeine	-	5.58
Catechin	47.78	196.20
Ellagic	73.41	-

Dedication types and concentrations of phenolic contents in avocado (fruit and seed) are recorded in Table (2). Data revealed that the type and concentrations of phenolic compounds in avocado (fruit and seed) by HPLC. Phenoilc acid (ppm) was the following: syrengic (2.16 and 768.59 ppm); coumarin, salycilic and Ellagic acid were only detected in avocado fruit; ferulic (4.5 and 14.60ppm); cinnamic (0.15 and 0.35ppm); pyrogallol (216.37and 225.73 ppm); protocatechuic (23.24 and 54.22 ppm); vanillic acid (3.76 and 50.78 ppm); catechol (20.96 and 28.82 ppm); catechin (47.78 and 196.20 ppm) and Caffeine was only detected in avocado seed.

Determination types and concentrations of flavonoids contents in avocado fruit is recorded in Table (3). Data revealed that the type and concentrations of flavonoids compounds in avocado fruit and seed) were rutin (81.06 and 52.97ppm); quercitrnic (86.70 and 112.54 ppm); luteolin and kampferol were detectable in avocado fruit; rosmarinic (60.52 and 113.89 ppm); quercitin (220.87and 62 ppm); hesperetrin (175.65 and 54.43 ppm); Apignen and hespererctin were only detectable in avocado seed.

Table (3): Types and concentrations of flavonoids compounds of avocado (fruit and seed) (ppm).

Flavonoids compounds	Avocado fruit	Avocado seed
Rutin	81.06	52.97
Quercitrinic	86.70	112.54
Luteolin	5.623	-
Kampferol	60.56	-
Rosmarinic	60.52	113.89
Quercitin	220.87	62
Hesperetin	175.65	54.43
Apignen	-	83.62
Hespererctin	-	385.87

Results recorded in Table (4) shows the effect of avocado (fruit and seed) at different levels on serum immunoglobulins (IgM and IgG) of rats. Results revealed that positive control group caused significant decrease ($P<0.05$) in the mean value of IgG and IgM compared to the negative control group. The addition of Avocado (fruit and seed) at (5 or 10%) significantly increased ($P<0.05$) the mean level of IgG and IgM compared to the positive control group. There was a significant difference ($P<0.05$) in the level of IgM between the two groups that fed on the different levels of Avocado (fruit or seed). In addition there was a significant difference ($P<0.05$) in the level of IgG between the two groups that fed on the different levels of Avocado fruit, while there was no significant difference ($P<0.05$) in the level of IgG between the two groups that fed on the different levels of Avocado seed. The higher supplementation with Avocado (fruit and seed), the higher immunoglobulins IgG and IgM level.

A result of Avocado (fruit and seed) at different levels on RBC, PCV, Hb and TLC concentrations of rats are noted in Table (5). Results revealed that positive control group had significant decrease (P<0.05) in the mean value of RBC, PCV and Hemoglobin in evaluation with the control healthy group, however the amount of TLC was significantly increased (P<0.05), in the positive control group. On the other hand, the supplementation with (5 and 10 % Avocado fruit or seed) significantly increased (P<0.05) the mean level of RBC, PCV and Hemoglobin but significantly decreased (P<0.05) the level of TLC compared to the positive control group. There were no significant distinctions in the amount of HB and TLC between two groups fed on different levels of Avocado fruit, but, there was a significant difference in RBC, PCV between groups given on both levels. In addition there are no significant differences (P<0.05) in the levels of RBC, HB and TLC between the two groups fed on the different levels of Avocado seed, but, there is a significant difference in PCV level between the groups fed on the two levels. The greatest increase in the relative red blood cell parameters concentrations was obtained by fed on supplemented diet with 10% of avocado (fruit and seed).

Table (4): Effect of avocado (fruits and seeds) supplementation on immunoglobulins of rats with induced immune deficiency

Parameters / Groups	IgG mg/ml	% of increment	IgM mg/ml	% of Change
Control (-ve)	5.34±0.24 ^{a}	-	3.67±0.18 ^{a}	-
Control (+ve)	1.52±0.07 ^{e}	-	1.13±0.13 ^{e}	-
Avocado Fruit (5%)	3.40±0.10 ^{c}	123.28	2.41±0.26 ^{c}	113.27
Avocado Fruit (10%)	3.85±0.05 ^{b}	153.28	2.99±0.10 ^{b}	164.60
Avocado Seed (5%)	2.58±0.08 ^{d}	69.73	1.78±0.02 ^{d}	57.52
Avocado Seed (10%)	3.09±0.09 ^{c}	103.28	1.93±0.06 ^{cd}	70.79

Values are expressed as means ± SE.

Values at the same column with different letters are significantly different at P<0.05.

Results illustrated in table (6) revealed the effect of Avocado fruit and seeds at different levels on liver functions of rats. The positive control had significant increase (P<0.05) in the mean value of serum AST, ALT, and ALP compared to the healthy control group. It could be observed that, these rats obtained significant reduction (P<0.05) in the mean value of serum albumin when compared to negative control group. However, the supplements based on a levels of Avocado fruit and seeds significantly decreased (P<0.05) the mean standard of serum liver intestinal enzymes compared to the control positive group. Even though got a significant increase (p<0.05) in serum standard of serum albumin compared to the same group. Moreover there are no significant changes (P≤0.05) in the amount of serum ALT, ALP and serum albumin between low and the high amount of Avocado fruit and seed), however there is significant changes in serum AST between two groups of Avocado fruits. The very best results of liver functions were recorded for group fed on supplemented diet with 10% of avocado (fruit and seed).

Table (5): Effect of Avocado (fruit and seed) supplementation a different levels on red blood cell parameters of rats

Parameters / Groups	RBCs (million/c.mm)	PCV %	Hemoglobin %	TLC (Th.c.mm)
Control (-ve)	5.70±0.20^{a}	55.15±0.55^{a}	14.25±0.15^{a}	4.90±0.90^{d}
Control (+ve)	3.15±0.05^{d}	30.75±1.25^{e}	8.40±0.90^{e}	13.10±0.80^{a}
Avocado Fruit (5%)	4.30±0.10^{c}	41.25±1.35^{c}	12.65±0.15^{bc}	7.75±0.35^{bc}
Avocado Fruit (10%)	4.80±0.10^{b}	49.90±1.20^{b}	13.95±0.15^{ab}	6.00±0.10^{cd}
Avocado Seed (5%)	4.15±0.15^{c}	35.30±0.40^{c}	11.15±0.35^{d}	9.58±0.58^{b}
Avocado Seed (10%)	4.35±0.15^{bc}	40.05±0.05^{d}	12.20±0.20^{cd}	9.15±0.55^{b}

Values are expressed as means ± SE. Values at the same column with different letters are significantly different at P≤0.05.

Table (6): Effect of Avocado (fruit and seed) supplementation at different levels on liver functions of rats

Parameters / Groups	AST	ALT	ALP	Albumin
	(µ/L)			(g/dl)
Control (-ve)	64.80±3.80^{d}	28.75±1.05^{d}	138.00±2.00^{e}	4.30±0.20^{a}
Control (+ve)	120.05±3.95^{a}	56.55±2.25^{a}	212.60±2.90^{a}	2.21±0.19^{c}
Avocado Fruit (5%)	96.10±4.10^{b}	34.45±2.65^{bc}	159.50±0.50^{cd}	3.85±0.15^{ab}
Avocado Fruit (10%)	70.15±7.35^{cd}	32.15±1.65^{bc}	151.00±4.00^{d}	4.05±0.15^{a}
Avocado Seed (5%)	100.15±4.45^{b}	42.05±0.65^{b}	176.05±4.95^{b}	3.35±0.14^{b}
Avocado Seed (10%)	84.60±0.70^{bc}	37.20±1.20^{bc}	165.65±4.35^{bc}	3.75±0.15^{ab}

Values are expressed as means ± SE. Values at the same column with different letters are significantly different at P≤0.05.

Effects illustrated in Table (7) revealed the effect of Avocado (fruit and seed) at different levels on lipid profile in rats. The positive control group acquired significant increase (P<0.05) in serum levels of TC, TG, VLDL-c, and LDL-c caused significant decrease in serum HDL-c, as compared to the healthy control group. On the other hand, the supplementation with different levels of Avocado fruit and seed (5and 10%) had significant decreased (P<0.05) in serum levels of TC, TG, VLDL-c, and LDL-c as compared to the positive control group, While had a significant increase (p<0.05) in serum level of serum HDL-c, compared to the same group. Additionally, there are a significant changes (P<0.05) in the levels of TC and LDL-c between low and the high levels of Avocado fruits , however there are no significant modifications in our levels of TG, HDL-c, and VLDL-c, between the same groups. Furthermore, There are a significant differences (P<0.05) in the levels of TG, and VLDL-c between the low and the high levels of Avocado seed , however there were no significant changes in the levels of TC, HDL-c, and LDL-c between same groups. The greatest decrease of lipid profile are noted for group fed on supplemented diet with 10% of avocado (fruit and seed).

Table (7): Effect of Avocado (fruit and seed) supplementation at different levels on lipid profile in rats

Parameters / Groups	TC	TG	HDL	VLDL-C	LDL-C
	(mg/dl)				
Control (-ve)	78.90±0.10^{e}	56.50±2.50^{d}	55.50±2.50^{a}	11.30±0.50^{d}	12.10±1.90^{c}
Control (+ve)	118.10±3.10^{a}	93.15±1.85^{a}	35.75±1.25^{d}	18.63±0.37^{a}	63.72±3.98^{a}
Avocado Fruit (5%)	96.70±2.40^{b}	75.40±3.60^{bc}	45.70±3.30^{bc}	15.08±0.72^{bc}	35.92±0.18^{b}
Avocado Fruit (10%)	85.15±2.15^{de}	65.00±4.00^{cd}	52.05±2.95^{ab}	13.00±0.80^{cd}	13.85±3.65^{c}
Avocado Seed (5%)	95.50±1.50^{bc}	80.50±3.50^{b}	43.65±1.35^{c}	16.10±0.70^{b}	35.75±0.85^{b}
Avocado Seed (10%)	88.20±2.20^{cd}	67.70±1.70^{c}	46.35±0.65^{bc}	13.54±0.34^{c}	28.31±3.19^{b}

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at $P<0.05$.

Discussion

Plant life are essential and important part in complementary and natural medicine. They develop a chance to form supplementary metabolites like alkaloids, flavonoids, steroids and phenolic chemicals which might be used to create health and cure diseases (**Spelman *et al.*, 2006**). Due to concerns about side effect of standard drugs, the use of natural products as an alternative to conventional treatment in healing and treatment of many diseases has been on the surge in the last many years. Medicinal plants provide as therapeutic alternatives, more secure choices or sometimes as the only effective treatment. Psychological data reports that many medicinal activities are related to the immune system stimulatory and antioxidant properties of plant secondary metabolites. Quite a lot of these plants and their remote constituents have shown beneficial effects such as antioxidant, anti-inflammatory, anticancer, antimicrobial and immunomodulatory results **(Miller, 2006)**. Supplementation with the antioxidant protected immunity process reactions in individuals exposed to certain environmental causes of free radicals (**Ivanov, 2008)**.

Though our bodies can activity antioxidant enzymes, we also need additional intake of dietary antioxidants to improve our immunity and protect all of us from the harmful results of free radicals and oxidative stress. Avocado (fruit and seed) have an increased nutritive value and antioxidant substances, however there is no documented evidence of any investigation of the immunomodulatory actions. Consequently, the key objective of this work was to investigate the effect of Avocado (fruit and seed) supplementation on immune system and other chemical parameters in rats.

Normally, the fruit is a rich source of bioactive compounds and could be used to develop value added companies other food applications to raise the health benefits. The obtained substances have powerful antimicrobial along with antioxidant properties and could play a component in drug development, product and spa (**Bhardwaj *et al.*, 2014)**.

The current results revealed that identified types of phenolic compounds found in avocado (fruit and seed) were syrengic, ferulic; cinnamic; pyrogallol protocatechuic; vanillic acid; catechol; and catechin. Coumarin, salycilic and Ellagic acid were only detected in avocado fruit;while caffeine was only detected in avocado seed (Table 2). These results are agreed with **Murakami *et al.*, (2002)** who mentioned that avocado fruit contains ferulic acid (FA) as a natural phenolic compound. Also **Shehata and Soltan, (2013)** reported that phenolic compound of avocado (fruit and seed) were pyrogallol; protocatechuic; vanillic acid; catechol; catechin. Syringic was only uncovered in avocado seed. Ellagic acid only detected in avocado fruit.

Wang *et al.*, (2010) and Gorinstein *et al.*, (2011) mentioned that avocado seed content the highest total phenolic content and antioxidant capabilities whereas the pulp acquired the minimum. **Rodriquez-Carpena *et al.*, (2011)** reported that phenolic substances are widely allocated in flesh and seeds avocado (caffeic acid, P-Hydroxy benzoic, chrouogenic, ferulic and epicatichin). Recently, **Pahua-Ramos *et al.*, (2012)** reported that protocatechuic acid was the main phenolic compound identified, followed by kaempferide and vanillic acid. In addition, clorogenic acid, syringic acid, rutin and kaempferol were present in small amounts.

Peterson *et al.*, (2012) reported that flavonoids are a family group band of phenolic chemicals with strong antioxidant activity within fruits, vegetables and other plant foods. The present results revealed that the identified types of flavonoids in avocado (fruit and seed) were Rutin, quercitrnic, rosmarinic, quercitin, hesperetrin, luteolin and kampferol were detectable in avocado fruit; while Apignen and hespererctin were only detectable in avocado seed (Table 3). These results were in accordance with **Shehata and Soltan, (2013)** who reported that flavonoids compound of avocado (fruit and seed) were Rutin, quercitrnic and quercetin, Apignen was only detectable in avocado seed ,avocado fruit and seed have higher content flavonoids and lower level of ascorbic acid. **Kosinska *et al.*, (2012)** indicated that the avocado seed and peel are rich in flavonoid (quercitin).

The addition of Avocado (fruit and seed) at different levels increased the concentrations of IgM and IgG in the rats. This enhancement of immune functions may be related to the chemical composition of Avocado fruit that contains a high levels of antioxidant compounds such as phenols, flavonoids, alkaloids, saponins, steroids tannins, phytosterols and vitamins (E, ascorbic acid, and Carotene)

Polyphenols of several sources have shown a modulator effect on epigenetic mechanisms as GENETICS methylation, histone modifications and posttranscriptional regulation by microRNAs, and these mechanisms in change, can modulate immune system influencing both activation and differentiation of multiple cellular types included in the immune system response (**Cuevas *et al.*, 2013)**.

Supplement Vitamin C is an essential water-soluble nutrient that generally exerts its effect on host immunity process and induces the immune system (**Pavlovic and Sarac, 2010)**.Consumption of diet containing vitamins E and C could help to ameliorate the oxidation effect caused by gasoline (as a free radical) increasing the haemoglobin concentration and decreasing white cell count **(Ibitoroko *et al.*, 2011)**. Ascorbic acid enhances antioxidant defenses of T-cells (**Pavlovic *et al.*, 2009)** and also increase T-cell responsiveness to antigens, suggesting a role in regulating immune system (**Wu *et al.*, 2000**). Supplement Vc was shown to improve individual immune response, such as antimicrobicidal, natural monster cell activities, lymphocyte expansion and chemotaxis (**Pavlovic *et al.*, 2005**), suggesting the most beneficial role of this supplement in regulating the immune system response.

Avocado (fruit and seed) in the current study caused a significant increase ($P<0.05$) in haematological indices PCV, RBC and Haemoglobin concentration of the treatment plan rats in comparison to the control positive group especially at higher levels. This is attributed to the free radical scavenging activities of flavonoids and other plant phenolics or to the metal-binding activity and immunity process stimulating properties of polyphenolics (**Middleton and Kandaswami, 1992)**.

The rise during these variables suggests a greater production of vast majority of the cells included in the immunity process which are produced in the stem skin cells of the bone marrow. This can be thought to have a stimulatory impact on the immune reactions since increased production of the immune system cells may imply a greater immune system function (**Sainis *et al.*, 1997**). Like a dietary component, flavonoids are thought to have health-promoting properties because of the high antioxidant capacity in vivo and in vitro systems (**Cook and Samman, 1996)**.

The obtained results revealed that, supplementation with different levels of Avocado (fruit and seed) decreased the elevated level of liver functions. These results agreed with **Shehata and Soltan, (2013)** who reported that, supplemented diet with different concentration (10, 20 and 30%) of avocado (fruit and seed) reduced the AST and ALT activities compared to positive control group. These results are in agreement with those reported by **Al-Dosari,(2011)**, who indicated that rats which consumed one or two ml/day/rat avocado extract for 70 days showed decrease in AST and ALT activity in comparison to the control group. Also, **Mahmoed and Rezq,(2013)** reported that administration of dried out avocado fruit at the three tests levels (5, 10 and 15%) induced lower of serum AST and ALT content in comparison to the control group.

The beneficial effect of avocado fruits may be attributed to its phenolic and flavonoids which have antioxidant properties. These results agreed with **Itoh *et al.*, (2010)** who indicated that, intravenous administration of syringic acid or vanillic acid significantly decreased the activities of AST and ALT. Therefore, syringic acid and vanillic acid might be promising oral agents for the prevention of liver disease. **Kawagishi *et al.*, (2001)** reported that avocado compounds might help in treating viral hepatitis. Simply because, these results proved with **Art *et al.*, (2009)** who reported that, the avocado contains several effective components such as alkaloids, saponins, flavonoids, steroids that reduce liver damage.

The obtained results exposed that, supplementation with different levels of Avocado (fruit and seed) decreased the increased standard of lipid profile in rats, while had significant embrace serum level of HDL-c. These results may be due to avocado fruits that are rich in monounsaturated fatty acid, dietary fiber, flavonoids, phenolic compound and sterols. These results are accordance with those reported by **Al-Dosari, (2011)**, who revealed that the avocado fruit pulp administrated at doses 1 and 2ml/day/rat for ten week caused a significant lower in the serum lipid including TC and TG levels and increased in HDL-c. **Kris-Etherton *et al.*, (1999)** reported that avocado is a fantastic source of monounsaturated fatty acids might actually lead to increased HDL cholesterol, lower triglycerides. These results agreed with **Perez and Garcia-Hernandez, (2007)** who indicated that the inclusion of avocado in the diet decreased plasma triglycerides, total lipid, LDL-c and VLDL-c and an increase in HDL-c. These results agreed with that reported by **Steven *et al.*, (2009)** who reported that one week diet based on avocado in hypercholesterolemia patients can dramatically decrease bad cholesterol (LDL) and triglyceride levels by 22%. Furthermore, good cholesterol (HDL) increased by 11%.

The lowering effect of avocado seed of TC, TG and LDL-c may be due to the avocado seed contains on phenolic compound and flavonoid compounds, soluble dietary fiber. These varieties of results agreement with (**Anderson, 1995)** mentioned that healthy fiber, especially soluble, effectively reduce serum cholesterol and LDL-c concentration. These results are in agreement with **Asaolu *et al.*, (2010**), who reported treatment hypercholesterolemic rat with assorted portions of methanolic of avocado seeds (50, 100 and 200 ml) caused an important reduction in the levels of TC, TG and LDL-c. **Hung *et al.*, (1997)** reported that flavonoid (quercetin, hesperidins' and narnigin) have been proven to inhibit the era or release of free radicals derived from lipoxygenase.

These results are in agreement with **(Harnafi *et al.*, 2009 and Kumer *et al.*, 2011)**, who reported that TC, TG and LDL-c showed significantly higher in hyper-cholesterolemic group than normal control group. Supplemented diet of hypercholesterolemic rats centered on a degree of avocado (fruit and seed) lead to significantly reduce P<0.05 in TG, TC and LDL-c concentration as well as significant increase in standard of good cholesterol HDL-c when compared to HC group. The decrease in TC, TG and LDL-c level and increases in HDL-c were increases with increasing concentration of avocado (fruit and seed).

Conclusion: It could be concluded that, Avocado (fruit and seed) supplementation generally showed immune stimulatory effect in rats due to the presence of phytochemicals like flavonoids and total phenols.

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التأثيرات العلاجية لفاكهة الأفوكادو وبذورها على نقص المناعة فى الفئران

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ملخص

الأفوكادو فاكهة تستخدم على نطاق واسع في العديد من البلدان لفوائدها الغذائية والطبية. أجريت هذه الدراسة لتقييم تأثيراعطاء فاكهة الأفوكادو وبذورها لمدة ستة أسابيع على وظائف الدم ، الجهاز المناعي ووظائف الكبد ودهون الدم في الفئران التى تعانى من نقص المناعة. ولقد أظهرت النتائج إحتواء المستخلص الميثانولي من فاكهة الأفوكادو وبذورها علي مركبات الفينول (258.75، 283.93 مجم GAE /100 جم)، الفلافونيد (2.89، 3.30 مجم CE /100 جم) ، قدرتها المضادة للأكسدة (89.7، 69.5%) وحامض الاسكوربيك (9.35 ، 5.22مجم /100 جم). تم تقسيم عدد 36 من ذكور الفئران البالغة من سلالة الألبينو الي ستة مجموعات، المجموعة الاولي وهي المجموعة الضابطة السالبة وتتغذي علي الغذاء الاساسي فقط . بينما الخمس مجموعات الأخري (6 فئران في كل مجموعة) تم حقنهم بدم الخراف لاحداث نقص فى المناعة . وتم تغذية احد هذه المجموعات علي الغذاء الأساسي فقط وهي تمثل المجموعة الضابطة الموجبة ، تم تغذية مجموعات (3،4) من الفئران على النظام الغذائي الأساسي المدعم مع (5 ، 10 ٪) من ثمار الأفوكادو على التوالي ، تم تغذية المجموعات (5،6) من الفئران على النظام الغذائي الأساسي المدعم مع بذور الأفوكادو (5 ، 10 ٪) على التوالي. أوضحت النتائج الي أن اعطاء الأفوكادو (الفاكهة والبذور) عند مستويات (5 ، 10٪) إلى الفئران أدي الي حدوث ارتفاع معنوى $P\leq0.05$ في قيم المناعة IgG , IgM بنسبة 123.28، 153.2، 69.73، 103,28%، 113.27، 164.60، 57.52، 70.79% علي التوالي مقارنة بالمجموعة الضابطة الموجبة. مقاييس الدم قد ارتفعت ارتفاع معنوى $P\leq0.05$ أيضا. علاوة علي ذلك ، قد تحسنت وظائف الكبد بدرجة ذات دلالة احصائية $P\leq0.05$ مقارنة بالمجموعة الضابطة الموجبة. بالإضافة إلى ذلك، فإن تأثير خفض (الفاكهة والبذور) على ملامح الدهون في الدم. وتوصى الدراسة الحالية باستخدام فاكهة وبذورالأفوكادو حيث ادى تناولهم إلى تنشيط المناعة لدى الفئران المصابة بنقص المناعة.