

## The Antioxidant Activity of Avocado seeds on Immunosuppression induced by Cyclosporine in Rats.

Suzan A. Saad

Assistant Professor of Nutrition and Food Science In Faculty of Home Economics, Al- Azhar University

### Abstract:

Avocado seeds are containing a variety of essential nutrients and important phytochemicals, exhibited potential antioxidant properties. This study aimed to assess the immune effect of avocado seeds against the Cyclosporine (CsA) immunosuppressive rat model. Twenty four male albino rats used in the experiment .Rats divided into equal 4 groups: (G1) was given basal diet as negative control group (-ve). (G2), received (CsA) (50 mg/kg/day subcutaneous in olive oil for 10 days) then given standard diet for 28 days as positive control group(+ve) .(G3) was fed on basal diet supplemented with avocado seeds powder (5%).(G4), received (CyA) (50 mg/kg/day subcutaneous in olive oil for 10 days) then given basal diet supplemented with avocado seeds powder (5%). The biological parameters were assessed including feed intake (FI), feed efficiency ratio (FER), and body weight gain (BWG). At the end of experimental period (28day) complete blood picture, Red blood cells (RBCs) and white blood cells (WBCs) were measured. Total serum immunoglobulins (IgG , IgA and IgM) and TNF- $\alpha$  were examined .Total protein and albumin were determined . Histopathological examination for spleen was done. Also the antioxidant enzymes (SOD,CAT and GPx) and MDA were assessed in spleen tissues The results indicated a significant decrease in BWG, FI, and FER in rats treated with CsA. Also showed that CsA caused decrease in RBCs, WBCs counts, hemoglobin, and platelets. CsA administration showed significant decrease in serum immunoglobulins and increase TNF- $\alpha$ . Furthermore increase in MDA and decrease in the antioxidant enzymes. Supplementation with avocado seeds powder with CsA ,improved activity of antioxidant enzymes in spleen tissue and serum immunoglobulins. The result indicated that, avocado seeds can improve the immune system against the CsA immunosuppressive rats.

**Key words :** Avocado, Phytochemicals, Antioxidants, Immunosuppressive, Cyclosporine.

## Introduction :

Cyclosporine (also known as Ciclosporin or Cyclosporin A) is acyclic peptide of 11 amino acid residues isolated from the fungus *Tolypocladium inflatum*. Cyclosporine (CsA) is the first immunosuppressant that was found to allow selective immune regulation of T cells without excessive toxicity (**Rezzani, 2004**). The introduction of cyclosporine has revolutionized organ transplantation. It is found to be highly effective in the prevention of transplant rejection, while leaving the functioning of the rest of the immune system largely intact. It is currently used to prevent graft rejection in the kidney, liver, heart, lung, combined heart-lung transplants, rejection following bone marrow transplantation, and also in the prophylaxis of host-versus-graft disease. Cyclosporine is also used in most autoimmune diseases apart from transplantation (**Magnasco et al., 2008**).

Many experimental evidence was reported that CsA induced imbalance between production of Reactive oxygen species (ROS) and antioxidant defense systems. Indeed when oxidative stress overcomes on antioxidant defense, lipid peroxidation progresses (**Shahaboddin et al., 2011**). The mechanism of action of cyclosporine is as a calcineurin inhibitor, a cytochrome P450 3A4 inhibitor, and P-glycoprotein inhibitor. Cyclosporin A (CsA) inhibits the synthesis of interleukins (IL), including IL-2, which is essential for self-activation T lymphocytes (LT) and their differentiation (**Pradier et al., 2019**).

Recently, fruit waste has become one of the main sources of municipal solid wastes, which have been an increasingly tough environmental issue. One of the solutions to this problem was directed to use fruit wastes as a source of valuable compounds; the bioactive constituents and using them in the food, pharmaceutical, as well as cosmetics industry. Thus, its utilization might be of considerable economic benefits and has become increasingly attractive (**Deng, et al., 2012**). The byproducts of fruits and vegetables used to make up of peels and seeds of different shapes and sizes that normally have no further usage and commonly wasted or discarded (**Varzakas et al., 2016**).

Avocado seeds might represent about 20% from its mass amounting as waste at a volume more than one million tons per year. This waste has a significant environmental impact due to the organic charge. It also would require additional costs for handling and storage (**Gómez et al., 2014**).

**Hurtado-Fernandez et al., (2011)** attracted the attention of using avocado seeds, due to their role in the plant's immune response, their influence in the oxidative stability and organoleptic characteristics of foods, and the wide

variety of health-promoting effects attributed to them. This waste might represent an ecological or human contaminant. On the other hand, data on its chemical composition could qualify it for use in food or animal feed. The avocado seeds found to be rich in phenolic compounds that could play a role in the putative health effects (**Dabas et al., 2013**). Several biological activities of the avocado seed have been reported such as antioxidant, antihypertensive, fungicidal, larvicidal, hypolipidemic, and recently amoebicidal and giardicidal activities (**Jiménez-Arellanes et al., 2013**). In addition, avocado seeds have high contents of bioactive phytochemicals, such as phenolic acids, condensed tannins, and flavonoids, including procyanidins, flavonols, hydroxybenzoic, and hydroxycinnamic acids (**Figuroa, et al., 2018**). The present study aimed to investigate the effectiveness of avocado seed powder supplements against the CsA immunosuppressive rat model.

## **Materials and Methods :**

### **Plant material, drug, chemical and biochemical kits**

- Fresh avocado fruits, corn oil and starch as well as olive oil used in this study were purchased from the local market Tanta City Gharbia Governorate, Egypt.
- Casein, cellulose, and choline chloride powder were obtained from Morgan Co. Cairo, Egypt.
- Animals: Twenty four mature male albino rats of *Sprague- Dawley* strain weighing between  $180 \pm 20$  g., were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.
- Kits were purchased from Egyptian American Company for Laboratory Service and Supplied by Alkan Company.
- Cyclosporine (CsA) was purchased from Sigma Chem. Co., St Louis, Mo. U.S.A.

## **Methods :**

### **Sample preparation:**

Avocado (*P. americana*) fruit used in the present study were obtained from the local market. The fruits at the onset of ripening were cut open to obtain their seeds. The seeds were washed then dried at  $250^{\circ}\text{C}$ . After drying, the samples were ground to fine powder and stored in dark airtight bottles in the refrigerator till use.

### Experimental design:

The animals were acclimatized for one week, fed basal diet according to **Reeves *et al.*,(1993)** and water supply *ad libitum*. Rats were randomized into four groups (6 rats each) :

**Group1:** Rats were given basal diet and served as negative control group (-ve). **Group 2 :** Rats received Cyclosporine (CsA) (50 mg/kg/day subcutaneous in olive oil for 10 days) then fed standard diet for 28 days and kept as positive control group (+ve). **Group 3:** Normal rats were fed on basal diet supplemented with avocado seeds powder (5%) for 28 days. **Group 4:** Rats received CsA (50 mg/kg/day subcutaneous in olive oil for 10 days) then fed basal diet supplemented with avocado seeds powder (5%) for 28 days.

At the end of experiment (28) days . The animals deprived from food and water overnight before being sacrificed. Blood samples were collected in dry centrifuge tubs from hepatic portal veins. Serum samples were separated by centrifugation at 4000 rpm for 10 minutes and kept in plastic vial at -20°C till analysis. **Spleen** removed, washed with isotonic saline, dried by filter paper and weight then divided into two samples. **The first sample** of spleen kept in formalin saline 10% for histopathological examination. **The second sample** kept at -80°C for preparation of tissue homogenate for determination of antioxidant and peroxide parameters. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was used for the assay of some laboratory analysis.

### Determination of some biological parameters:

During the experimental period, the diet consumed was recorded every day, and body weight recorded every week. The body weight gain (BWG%) and feed efficiency ratio (FER) were determined according to **Chapman *et al.*, (1959)** .

### Determination of differential complete blood counts:

Hemoglobin concentrations, total and differential white blood cells(WBC), red blood cell(RBCs), and platelet counts were done. All counts were done manually using a hemocytometer (Celeromics Company Cambridge, UK) and appropriate stains. For the determination of hemoglobin concentration, the Drabkin's cyanmet hemoglobin reagent was used.

### **Biochemical analysis of serum:**

Serum immunoglobulins (IgG , IgA and IgM) were estimated by enzyme linked immune sorbent assay (ELISA) according to **Burlingame and Rubin, (1990)**. Albumin (**Drupt, 1974**) and total protein ( **Sonnenwirth and Jaret, 1980**)

### **Assessment of Oxidant/Antioxidant Activity:**

Supernatant were used for estimation of different antioxidant level by colorimetric method using spectrophotometer (Merck thermo spectronic, Model NO. UV-1, double beam), Lipid peroxidation by malondialdehyde (**Draper and Hadley,1990**), Superoxide dismutase (SOD) by method developed by **Misra and Fridovich (1972)** and Catalase (CAT) by colorimetric assay (**Sinha,1972**). GP<sub>x</sub> was determined using the procedure of ( **Ellman, 1959**)

### **Histopathological examination:**

For microscopic evaluation, spleen samples were fixed in a fixative (formalin solution neutral buffered, 10%) and embedded in paraffin sectioned at 5 m thickness. The sections were subsequently stained with Mayers' hematoxylin and eosin. The stained samples were examined under light microscope at 10× magnification. The Slides were photographed, and documented (**Bancroft *et al.*, 1996**).

### **Statistical analysis:**

All the obtained data was be statistically analyzed by SPSS computer software. The calculated occurred by analysis of variance ANOVA according to **Armitage and Berry, (1987)**.

### **Results :**

Table(1) showed that; the body weight gain percent(BWG%), feed intake(FI), feed efficiency ratio (FER) and spleen weight/ body weight were significantly( $p < 0.050$ ) lowered in the toxic CsA group (+ve) when compared with the negative control(-ve). Also there were a significant decrease in avocado seeds alone group compared with the (-ve) control group. The avocado seeds treated group with CsA recorded a significant increase for above parameters when compared with the (+ve) group .

**Table (1)** :Effect of avocado seeds powder on change in body weight gain percent, feed intake, feed efficiency ratio , and spleen weight of rats with cyclosporine-induced immunodeficiency

Parameter Group	BWG % M±SD	FI (g/day) M±SD	FER M±SD	Spleen weight(g) M±SD
Normal control (-ve)	39.96±3.38 <sup>a</sup>	17.83±0.22 <sup>a</sup>	0.11±0.01 <sup>a</sup>	0.70±0.08 <sup>a</sup>
Cyclosporine (+ve)	19.49±2.32 <sup>c</sup>	12.53±0.05 <sup>c</sup>	0.080±0.00 <sup>c</sup>	0.50±0.08 <sup>c</sup>
Avocado seeds	30.33±3.14 <sup>b</sup>	16.92±0.77 <sup>b</sup>	0.086±0.00 <sup>c</sup> <sup>b</sup>	0.73±0.05 <sup>a</sup>
Cyclosporine+ avocado seeds	26.26±4.69 <sup>b</sup>	12.63±0.13 <sup>c</sup>	0.10±0.01 <sup>b</sup>	0.63±0.05 <sup>b</sup>

Significance is expressed at  $p < 0.05$  using one way ANOVA test. - Values which have different letters in each column differ significantly.

**Table (2)**:Illustrated the mean and standard deviation of hemoglobin, RBCs count, and mean cell volume (MCV) of rats. There were significant decrease in the toxic CsA group when compared with the (-ve) control. While, there were significant increase in the mean value of cell hemoglobin , RBCs count, and mean cell volume (MCV) in avocado seeds treated group with CsA. The best results showed by the group treated with avocado seeds only.

**Table 2**:Effect of avocado seeds powder on hemoglobin, RBCs and MCV of rats with cyclosporine-induced immunodeficiency

Parameter Group	Hemoglobin (g/dl) M±SD	RBCs count (10 <sup>6</sup> /mL) M±SD	MCV F/L M±SD
Normal control (-ve)	15.6±0.90 <sup>a</sup>	4.88±0.09 <sup>a</sup>	78.56±1.27 <sup>a</sup>
Cyclosporine (+ve)	9.35±0.59 <sup>d</sup>	2.76±0.15 <sup>d</sup>	62.03±1.26 <sup>d</sup>
Avocado seeds	12.44±0.49 <sup>b</sup>	4.6±0.16 <sup>b</sup>	76.06±1.8 <sup>b</sup>
Cyclosporine + avocado seeds	11.25±0.97 <sup>c</sup>	3.86±0.11 <sup>c</sup>	71.99±6.25 <sup>c</sup>

Significance is expressed at  $p < 0.05$  using one way ANOVA test. - Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

**Table (3)** revealed that; mean of WBC count, Lymphocytes (LYM), Monocytes and Platelets of (+ve) control group recorded a significant decrease

compared with (-ve) control . On the other hand there were a significant increase in avocado seeds group and avocado seeds treated group with CsA. The group treated with avocado seeds only showed the best results for all groups.

**Table3.** Effect of avocado seeds powder on hematology (WBC) of rats with cyclosporine-induced immunodeficiency

Parameter Group	WBC count Thousand/ $\mu$ m M $\pm$ SD	Lymphocytes (%) M $\pm$ SD	Monocytes (%) M $\pm$ SD	Platelets Thousand/ $\mu$ m M $\pm$ SD
Normal control (v-)	6.22 $\pm$ 0.36 <sup>b</sup>	81.35 $\pm$ 0.66 <sup>a</sup>	1.38 $\pm$ 0.12 <sup>b</sup>	1073 $\pm$ 107.7 <sup>b</sup>
Cyclosporine (v+)	1.63 $\pm$ 0.11 <sup>d</sup>	50.600 $\pm$ 0.08 <sup>c</sup>	0.80 $\pm$ 0.04 <sup>d</sup>	715.66 $\pm$ 33.15 <sup>d</sup>
Avocado seeds	6.74 $\pm$ 0.02 <sup>a</sup>	81.35 $\pm$ 0.66 <sup>a</sup>	1.62 $\pm$ 0.06 <sup>a</sup>	1150 $\pm$ 44.72 <sup>a</sup>
Cyclosporine+ avocado seeds	4.6 $\pm$ 0.12 <sup>c</sup>	67.63 $\pm$ 2.25 <sup>b</sup>	1.10 $\pm$ 0.08 <sup>c</sup>	863.33 $\pm$ 11.25 <sup>c</sup>

Significance is expressed at  $p < 0.05$  using one way ANOVA test. - Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

### •Serum immunoglobulins, IgAs, IgMs and IgGs

**Table (4)** revealed that; mean value of IgAs, IgMs and IgGs were significantly decreased in the toxic CsA group (+ve) as compared to normal control group (-ve). But there were a significant increase in the levels of these parameters for treated group with avocado seeds powder with CsA. Also there was a significant increase for avocado seeds group. In the same table cyclosporine administration caused a significant elevation for tumor necrosis factor alpha (TNF- $\alpha$ ) , when compared to control group (-ve). On the other hand, treatment with avocado seeds powder significantly decreased ( $P < 0.05$ ) TNF- $\alpha$  compared with the CsA alone group.

**Table (4):** Effect of avocado seeds powder on serum IgA, IgM, IgG and TNF- $\alpha$  levels of rats with cyclosporine-induced immunodeficiency

Parameter Group	IgA (ng/ml) M $\pm$ SD	IgM (ng/ml) M $\pm$ SD	IgG(ng/ml) M $\pm$ SD	TNF- $\alpha$ M $\pm$ SD
Normal control (-ve)	0.98 $\pm$ 0.09 <sup>b</sup>	0.96 $\pm$ 0.04 <sup>b</sup>	1.47 $\pm$ 0.17 <sup>a</sup>	0.11 $\pm$ 0.09 <sup>c</sup>
Cyclosporine (+ve)	0.34 $\pm$ 0.01 <sup>d</sup>	0.34 $\pm$ 0.03 <sup>d</sup>	0.55 $\pm$ 0.01 <sup>c</sup>	0.64 $\pm$ 0.06 <sup>a</sup>
Avocado seeds	1.09 $\pm$ 0.09 <sup>a</sup>	1.006 $\pm$ 0.01 <sup>a</sup>	1.37 $\pm$ 0.20 <sup>a</sup>	0.09 $\pm$ 0.08 <sup>c</sup>
Cyclosporine+ avocado seeds	0.52 $\pm$ 0.017 <sup>c</sup>	0.66 $\pm$ 0.007 <sup>c</sup>	0.92 $\pm$ 0.01 <sup>b</sup>	0.18 $\pm$ 0.08 <sup>b</sup>

Significance is expressed at  $p < 0.05$  using one way ANOVA test. - Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

Table (5) showed that; the mean value of total protein, albumin and globulin in (+ve) control group recorded a significant decrease as compared to (-ve) control group. On the other hand, there were a significant increase in rats treated with avocado seeds only or avocado seeds treated with CsA group .

**Table5:** Effect of avocado seeds powder on total protein, albumin and globulin of rats with cyclosporine-induced immunodeficiency

Parameter Group	Total protein(g/L) M $\pm$ SD	albumin (g/L) M $\pm$ SD	Globulin(g/L) M $\pm$ SD
Normal control (-ve)	6.78 $\pm$ 0.18 <sup>a</sup>	3.63 $\pm$ 0.14 <sup>b</sup>	3.15 $\pm$ 0.06 <sup>a</sup>
Cyclosporine (+ve)	4.05 $\pm$ 0.15 <sup>c</sup>	2.26 $\pm$ 0.09 <sup>d</sup>	1.79 $\pm$ 0.205 <sup>c</sup>
Avocado seeds	6.73 $\pm$ 0.13 <sup>a</sup>	3.80 $\pm$ 0.08 <sup>a</sup>	2.93 $\pm$ 0.13 <sup>b</sup>
Cyclosporine + avocado seeds	6.06 $\pm$ 0.18 <sup>b</sup>	3.27 $\pm$ 0.09 <sup>c</sup>	2.79 $\pm$ 0.075 <sup>b</sup>

Significance is expressed at  $p < 0.05$  using one way ANOVA test. - Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

Table (6) cleared that, treatment of rats with CsA (+ve) group ;significantly induced lipid peroxidation in the spleen as indicated by an increase in the by-product of lipid peroxidation malondialdehyde. On the other hand, lipid peroxidation is significantly attenuated in animals treated with CsA in

combination with avocado seeds powder. Meanwhile; treatment with avocado seeds significantly increased all antioxidant enzymes levels ( $P < 0.05$ ) in spleen tissue compared with CsA alone or avocado seeds treated with CsA groups. Avocado seeds group recorded the best results for all groups

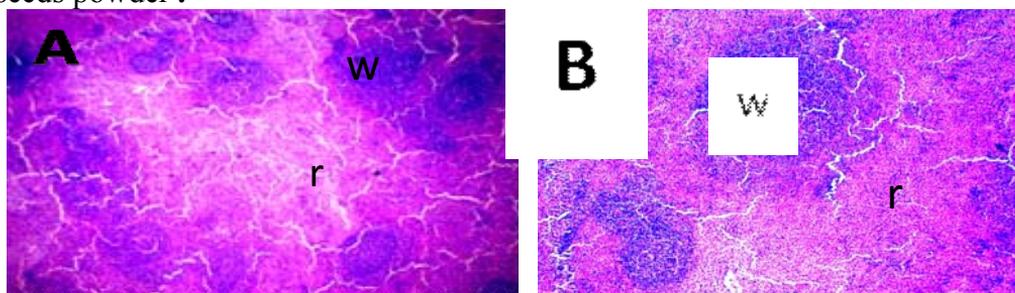
**Table 6:** Effect of avocado seeds powder on malondialdehyde and some antioxidant enzymes levels in the spleen homogenate of rats with cyclosporine-induced immunodeficiency

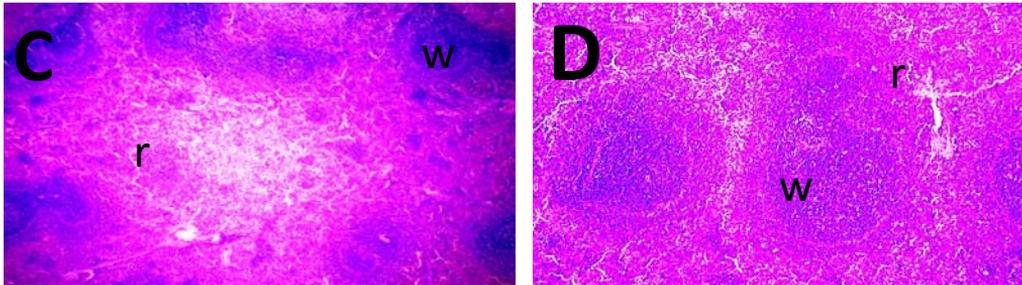
Parameter Group	MDA(nmol/mg protein)	SOD(U/min/mg protein)	CAT(U/min/mg protein)	GP <sub>x</sub> (ug/mg protein)
Normal control(-ve)	0.11±0.01 <sup>c</sup>	0.35±0.05 <sup>b</sup>	0.36±0.01 <sup>b</sup>	0.37±0.08 <sup>b</sup>
Cyclosporine (+ve)	0.40±0.01 <sup>a</sup>	0.10±0.012 <sup>d</sup>	0.11±0.08 <sup>d</sup>	0.11±0.01 <sup>d</sup>
Avocado seeds	0.07±0.08 <sup>d</sup>	0.38±0.08 <sup>a</sup>	0.38±0.01 <sup>a</sup>	0.40±0.08 <sup>a</sup>
Cyclosporine + avocado seeds	0.19±0.09 <sup>b</sup>	0.28±0.07 <sup>c</sup>	0.29±0.10 <sup>c</sup>	0.29±0.11 <sup>c</sup>

Significance is expressed at  $p < 0.05$  using one way ANOVA test. - Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

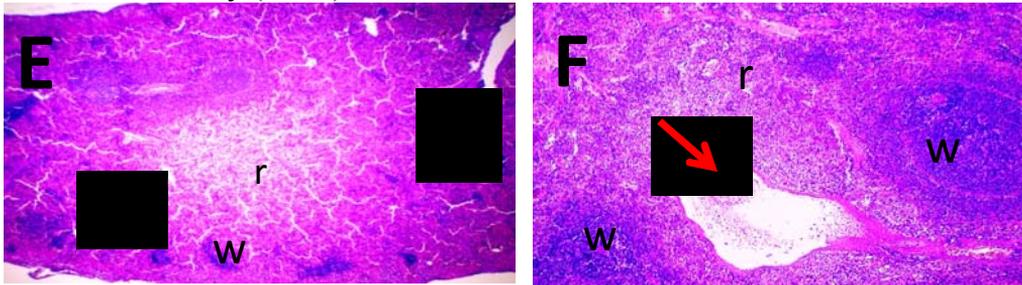
**Histopathological examinations:**

Spleen seeds powder sections obtained from normal rats as normal control (Untreated) and rats pretreated with cyclosporine then treated with diets supplemented with avocado seeds powder .

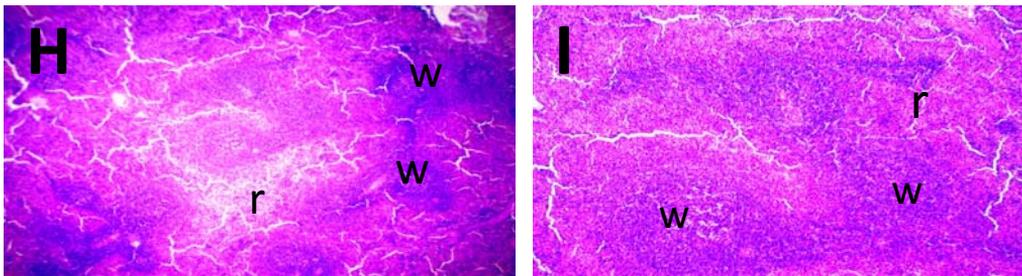




**Photo.1:** Microscopic pictures of H&E stained splenic sections showing normal distinct white (w) and red (r) pulps in control (-ve) group (A&B) and group received seeds only (C&D).



**Photo.2:** Splenic sections from control +ve group showing diffuse ill-defined white pulps (black arrows), congested blood vessel in red pulp (red arrow) (E-F).



**Photo.3 :** Splenic sections from group received drug and seeds showing improved histologic picture (H&I).

## Discussion

This study investigated the effectiveness of avocado seed powder supplements against the CsA immunosuppressive rat model. Cyclosporine (CsA) is an immunosuppressant drug widely used in organ transplantation to prevent rejection in people with organ transplants. In spite of its therapeutic effects, it has many adverse effects, such as anemia, thrombocytopenia, and increased liability to various infections. It reduces the immune system activity by interfering with the activity and growth of T-cell and decreasing the production of lymphocytes (Rezzani *et al.*, 2001). CsA administration was shown to be associated with a significantly lowered level of food consumption with a corresponding decrease in body weight. This is consistent with the findings of (Zizhang *et al.*, 2014). Since weight gain or loss is determined by a balance between dietary/food intake and energy expenditure, the reduction in body weight can be attributed to the recorded decrease in food consumption. Evidence exist on easy diffusion of CsA across the blood-brain barrier, thus having consequences on the nervous system. CsA may have suppressed the feeding centre in the lateral hypothalamus with a consequent inhibition of appetite (Katherine *et al.*., 2008). The important attenuating impact of avocado seeds on the alteration of eating behavior and body weight caused by medications indicate that avocado is known to be a superfood. It's giving health-nutrients to the body. However, there are almost 20 vitamins and minerals in avocado seed. It is also highly unsaturated fats such as monounsaturated and polyunsaturated fats (75 %). These ratings can influence feed intake and growth output in part, particularly when dose-dependent effects occur (Leite *et al.*, 2009). The reduction in relative spleen weight that was associated with CsA administration can be attributed to the, resulting in a marked decrease in the cellularity of periarterial lymphatic sheaths. The congested blood sinusoids in both the white and red pulp were the prominent feature in most of the examined sections together with thickened trabeculae. These results agree with Hala *et al.*, (2019) who studied the biological and hematological effects of *echinacea purpurea* L. roots extract in the immunocompromised rats with cyclosporine. The improvement in spleen weight in treated groups due to the major components of avocado were considered as anti-inflammatory compounds with both antioxidant and analgesic activities (Dinubile, 2010). In similar study, Pahua-Ramos *et al.*,

(2012) reported that the seed contained the high amount of phenols. **Arukwe et al., (2012)** suggested that phenols detected in parts of *P. americana* could further indicate their ability to act as anti-inflammatory, anti-clotting, antioxidants, immune enhancers. Immunosuppressive drugs play an important role in anemia. Reduction in the red blood cell counts, hemoglobin concentration, and the white blood cells (WBCs), with a decrease in the platelet counts, were observed in rats treated with CsA. These results agree with **Pally et al., (2001)** who found that administration of CsA (30 mg/kg) and ketoconazole (10 mg/kg) drugs induced significantly decrease in total WBCs and lymphocyte counts compared to control animal. Another study revealed that the treatment with cyclosporine daily doses (low, 2 mg/kg; medium 5 mg/kg, and high 10 mg/kg) for 31 days suppressed white cells count and lymphocytes, also inhibited cell-mediated immunity in dose-dependent manner compared with untreated group (**Lekhooa , 2015**). This reduction was previously explained by the reduced erythropoietin production, resulting in decreased stimulation of bone marrow erythropoiesis (**Nielsen et al., 2008**). On the other hand in the present study, the avocado supplementation with CsA administration improved cell hemoglobin, RBCs count, MCV, WBCs count, LYM, Monocytes and Platelets of rats. These findings were in accordance with those reported previously by **Walaa, (2019)**. This improvement attributed to the contents of avocado seeds as cichoric acid and echinacin, where each stimulates bone marrow and hematopoietic stem cells as mentioned in a previous study by (**Goel et al., 2002**). Also there were a significant decrease in mean value of IgA, IgM, IgG and increase in TNF- $\alpha$  levels for rats treated with CsA. The mechanism of action of cyclosporine is as a calcineurin inhibitor, a cytochrome P450 3A4 inhibitor, and P-glycoprotein inhibitor. CsA inhibits the synthesis of interleukins (IL), including IL-2, which is essential for self-activation T lymphocytes and their differentiation (**Pradier et al., 2019**). The avocado supplementation improved in mean value of IgAs, IgMs and IgGs for rats. Our results agree with **Fachinello et al., (2018)** who declared that the elevation of IgG production was resulted by supplementing some phytochemical to diet. The stimulation of phytochemical on IgG production has been observed in other study carried out by **Luo and Wu, (2011)**, which showed that phytochemical increased blood levels of IgA, IgG and IgM, improving the immunity of rats with cancer. Avocado seeds powder treatment

significantly increased total protein and albumin . This influence has been due to the seed's phenolic content, antioxidant activity and dietary coarse fiber content. Treatment of rats with CsA induced lipid peroxidation in the spleen as indicated by an increase in the by-product of lipid peroxidation MDA. CsA increased production of free radicals and oxidative stress. ROS can be produced either directly from CsA or during its metabolism by the cytochrome P450 (Cyt P450 3A) system (**Ahmed *et al.*, 1995**). Previous studies showed that CsA increased lipid peroxidation products, decreased glutathione in the liver and kidney of rats and impaired antioxidant defense system (**Capasso *et al.*, 2008**). Actually we found that treatment with avocado seeds powder significantly reduced MDA production and elevate both of, SOD, CAT and GP<sub>x</sub> in spleen . Polyphenols are in this sense one of the most essential bioactive compounds found in avocado seeds. In addition, avocado seeds have high contents of bioactive phytochemicals, such as phenolic acids, condensed tannins, and flavonoids, including procyanidins, flavonols, hydroxybenzoic, and hydroxycinnamic acids (**Figueroa, *et al.*, 2018**). Finally, the current findings revealed that treatment with avocado seeds powder ameliorated the hematological changes. However, there was a partial correction of the CsA-induced microscopic changes of the rat spleen. Histopathologically, these results agree with **Hala *et al.*, (2019)** who found that CsA induced a marked decrease in the cellularity of the white pulp with congested blood sinusoids of the red pulp together with significant depletion of periarteriolar lymphoid sheath.

## Conclusion

Avocado seeds have high contents of bioactive phytochemicals, such as phenolic acids, condensed tannins, and flavonoids, including procyanidins, flavonols, hydroxybenzoic, and hydroxycinnamic acids which improve the immune system against the CsA immunosuppressive rat model.

## References

**Ahmed, SS.; Napoli, KL and Strobel, HW.**(1995):Oxygen radical formation during cytochrome P450-catalyzed cyclosporine metabolism in rat and human liver microsome at varying hydrogen ion concentrations.Mol Cell Biochem;151:131–40.

**Armitage, P. and Berry, G.** (1987):Statistical Methods in Medical, Research.Blackwell Oxford, UK, pp. 93-213.

**Arukwe, U; Amadi, B. A; Duru, M.K.C; Agomuo, E. N; Adindu, E. A; Odika, P. C; Lele, K.C; Egejuru, L. and Anudike, J.** (2012):Chemical composition of *Persea americana* leaf, fruit and seed. IJRRAS, 11(2) 346-349.

**Bancroft, J.D.; Stevens, A. and Turner, D.R.** (1996): Theory and Practice of Histopathological Technique. 4th Ed., Churchill Livingstone.NY, London and Madrid.

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

**Capasso, G; Di Gennaro, C. and Della, Ragione F.**(2008): In vivo effect of the natural antioxidant hydroxytyrosol on cyclosporine nephrotoxicity in rats. Nephrol Dial Transplant; 23:1186–95.

**Chapman, D. G; Gastilla, R. and Cambell, J.A.** (1959):Evaluation of protein in food. LA. method for the determination of protein efficiency ratio. Can. J. Biochem. Phosiol, 37:679-686.

**Dabas, D., Shegog, R., Ziegler, G. and Lambert, J.** (2013): Avocado (*Persea americana*) seed as a source of bioactive phytochemicals. Current pharmaceutical design, 19(34): 6133-6140.

**Deng, G. F.; Shen, C., Xu, X. R., Kuang, R. D.; Guo, Y. J., Zeng, L. S., Gao, L.L.; Lin, X.; Xie, J.F.; Xia, E.Q. and Li, S.** (2012). Potential of fruit wastes as natural resources of bioactive compounds. International journal of molecular sciences, 13(7): 8308-8323.

**DiNubile, N. A.** (2010). A potential role for avocado and soybean-based nutritional supplements in the management of osteoarthritis: A review. The Physician and sports medicine, 38(2):71-81.

**Draper**, H.H. and **Hadley**, M. (1990): Malondialdehyde determination as index of lipid peroxidation. *J. Meth. Enzymol.*, 186: 421-431.

**Drupt**, F. (1974): Colorimetric method for determination of albumin. *Pharm.Biol.*, 9: 777-779.

**Ellman**, G.L. (1959): Tissue sulphhydryl groups. *J. Arich Biochem. Biophys.*, 82: 70-77.

**Fachinello**, M. R.; **Fernandes**, N. L. M., de **Souto**, E. R., dos **Santos**, T. C., da **Costa**, A. E. R., and **Pozza**, P. C. (2018): Lycopene affects the immune responses of finishing pigs. *Italian Journal of Animal Science*, 17(3): 666-674.

**Figuroa**, J. G., **Borrás-Linares**, I., **Lozano-Sánchez**, J. and **Segura-Carretero**, A. (2018): Comprehensive identification of bioactive compounds of avocado peel by liquid chromatography coupled to ultra-high-definition accurate-mass Q-TOF. *Food chemistry*, 245: 707-716.

**Goel**, V; **Chang**, C; **Slama**, JV; **Barton**, R; **Bauer**, R and **Gahler**, R,(2002): Alkylamides of *Echinacea purpurea* stimulate alveolar macrophage function in normal rats. *Int Immunopharmacol*:2:381-7.

**Gómez**, F. S; **Sánchez**, S. P; **Iradi**, M. G. G; **Azman**, N. A. M. and **Almajano**, M. P. (2014): Avocado seeds: Extraction optimization and possible use as antioxidant in food. *Antioxidants*, 3(2): 439-454.

**Hala**, A.H; **Khattab**; **Seham**, K; **Abounasef** and **Haneen**, L. **Bakheet**.(2019): The Biological and Hematological Effects of *Echinacea purpurea* L. Roots Extract in the Immunocompromised rats with cyclosporine. *J Microsc Ultrastruct.*, 7(2): 65-71.

**Hurtado-Fernandez**, E., **Pacchiarotta**, T., **Gomez-Romero**, M., **Schoenmaker**, B., **Derks**, R., **Deelder**, A. M., **Mayboroda**, O.A., **Carrasco-Pancorbo**, A. and **Fernandez-Gutierrez**, A. (2011): Ultra high performance liquid chromatography-time of flight mass spectrometry for analysis of avocado fruit metabolites: Method evaluation and applicability to the analysis of ripening degrees. *Journal of Chromatography A*, 1218(42):7723-7738.

**Jiménez-Arellanes**, A; **Luna-Herrera**, J; **Ruiz-Nicolás**, R; **Cornejo-Garrido**, J., **Tapia**, A. and **Yépez-Mulia**, L. (2013): Antiprotozoal and antimycobacterial activities of *Persea americana* seeds. *BMC complementary and alternative medicine*, 13(1): 109.

**Katherine, A.S;** **Niamh, M.M.** and **Steve, R.B.**(2008): Hypothalamic regulation of appetite, *Expert Rev. Endocrinol. Metab.* 3: 577–592.

**Leite, GJJ;** **Brito, EHS;** **Cordeiro, RA;** **Brilhante, RSN;** **Sidrim, JJC;** **Bertini LM,** (2009): Chemical composition, toxicity and larvicidal and antifungal activities of *Persea americana* (avocado) seed extracts. *Rev Soc Bras Med Trop.* ,42:110–3.5.

**Lekhooa, M.**(2015): Development of a Model to Characterize the Effect of Phela on Selected Immune Markers in Immune-Suppressed rats. University of the Free State, Pharmacology.

**Luo, C;** and **Wu, X. G.** (2011): Lycopene enhances antioxidant enzyme activities and immunity function in N-Methyl-N'-nitro-N-nitrosoguanidine-induced gastric cancer rats. *International journal of molecular sciences,* 12(5):3340-3351.

**Magnasco, A;** **Rossi, A;** **Catarsi, P;** **Gusmano, R;** **Ginevri, F.** and **Perfumo, F,** (2008): Cyclosporin and organ specific toxicity: Clinical aspects, pharmacogenetics and perspectives. *Curr Clin Pharmacol* ;3:166–73.

**Misra, H.P.** and **Fridovich, I.**(1972): The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.*;247(10):3170-5.

**Nielsen, FT;** **Jensen, BL;** **Marcussen, N;** **Skøtt, O.** and **Bie, P.**(2008): Inhibition of mineralocorticoid receptors with eplerenone alleviates short-term cyclosporin a nephrotoxicity in conscious rats. *Nephrol Dial Transplant*:23:2777–83

**Pahua-Ramos, M. E;** **Ortiz-Moreno, A.;** **Chamorro-Cevallos, G;** **Hernández-Navarro, M. D;** **Garduño-Siciliano, L;** **Necoechea-Mondragón, H.** and **Hernández-Ortega, M.** (2012): Hypolipidemic effect of avocado (*Persea americana Mill*) seed in a hypercholesterolemic mouse model. *Plant foods for human nutrition,* 67(1): 10-16.

**Pally, C.;** **Tanner, M;** **Rizvi, H;** **Papageorgiou, C .** and **Schuurman ,HJ.** (2001): Tolerability profile of sodium mycophenolate (ERL080) and mycophenolate mofetil with and without cyclosporine (Neoral) in the rat. *Toxicology.* :157:207–15.

**Pradier, A;** **Papaserafeim, M;** **Li N;** **Rietveld, A;** **Kaestel, C;** **Gruaz, L;** **Vonarburg, C;** **Spirig, R;** **Puga Yung, GL;** **Seebach JD.** (2019): Small-

Molecule Immunosuppressive drugs and therapeutic immunoglobulins differentially inhibit NK cell effector functions *in vitro*. front immunol. ;10:556.

**Reeves, P; Rossow, K. and Lindlauf, J.**(1993): Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. J. Nutr. 123: 1923-1931.

**Rezzani, R; Rodella; L. and, Bianchi, R.**(2001): Melatonin antagonises the cyclosporine A immunosuppressive effects in rat thymuses. Int Immunopharmacol. :1:1615–9.

**Rezzani, R.** (2004): Cyclosporine A and adverse effects on organs: histochemical studies. Prog Histochem Cytochem :39:85–128.

**Shahaboddin, ME; Pouramir, M. and Moghadamnia, A.A,** (2011):*Pyrus bioessieriana* Buhse leaf extract: An antioxidant, antihyperglycaemic and antihyperlipidemic agent. Food Chem. ;126:1730–3.

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

**Sonnenwirth, A. and Jaret, L.** (1980):Grad Wholes Clinical Laboratory Methods and Diagnosis. Vol.18th Ed Mosby, London ,258-259.

**Varzakas, T., Zakynthinos, G. and Verpoort, F.** (2016): Plant food residues as a source of nutraceuticals and functional foods. Foods, 5(4): 88.

**Walaa, S. Amin.**(2019): Effects of avocado (*Persea americana*) seed on Levels of Immune Cells and Body and Organs Weights in Mice. Sixth Scientific Conference (Fourth International) The Future of Quality Education and People with Special Needs in Light of the Concept of Quality.,1-16.

**Zizhang, O; Weiwei, C; Shaohua, Z; Xiaoping, L; Zhihua, Z; Xiangmao, Huirong; L. ; Sheng, J. and Yan, W.** (2014): Protective effects of 2- deoxy-D-glucose onnephrotoxicity induced by cyclosporine A in rats, Int. J. Clin. Exp. Path. ,7:4587–4595.

## الملخص العربي

النشاط المضاد للأكسدة لبذور الأفوكادو على تثبيط المناعة  
الناجم عن السيكلوسبورين في الجرذان.

تحتوي بذور الأفوكادو على مجموعة متنوعة من العناصر الغذائية الأساسية والمواد الكيميائية النباتية الهامة ، والتي تظهر خصائص محتملة مضادة للأكسدة. هدفت الدراسة الحالية إلى تقييم التأثير المناعي لبذور الأفوكادو ضد نموذج الفئران المثبط للمناعة عقار السيكلوسبورين . تم استخدام أربعة وعشرين فأراً ألبينو في التجربة. قسمت الجرذان إلى ٤ مجموعات: (مج ١) ، أعطيت النظام الغذائي القياسي كمجموعة ضابطة سلبية ، (مج ٢) حصل على السيكلوسبورين (٥٠) مجم / كجم / يوم تحت الجلد في زيت الزيتون لمدة ١٠ أيام) ، ثم أعطي النظام الغذائي القياسي لمدة ٢٨ يوماً كمجموعة ضابطة إيجابية. (مج ٣) علي الوجبة القياسية مدعمة بمسحوق بذور الأفوكادو (٥٪). (مج ٤) حقنت بالسيكلوسبورين (٥٠) (مجم / كجم / يوم تحت الجلد بزيت الزيتون لمدة ١٠ أيام) ثم أعطيت الوجبة القياسية مدعمة بمسحوق بذور الأفوكادو (٥٪). تم تقييم المؤشرات البيولوجية بما في ذلك الطعام المتناول ، ونسبة كفاءة التغذية ، وأوزان الجسم. في نهاية الفترة التجريبية (٢٨ يوماً) تم قياس صورة الدم الكاملة ، تم قياس خلايا الدم الحمراء (كرات الدم الحمراء) وخلايا الدم البيضاء. تم فحص الجلوبيولين المناعي الكلي في الدم والأجسام المناعية وتم تحديد البروتين الكلي والألبومين. تم فحص الأنسجة في الطحال. أشارت النتائج إلى انخفاض معنوي في الطعام المتناول ، ونسبة كفاءة التغذية ، وأوزان الجسم في الفئران المعالجة بالسيكلوسبورين. وأظهرت أن السيكلوسبورين يقلل من عدد كرات الدم الحمراء وعدد كرات الدم البيضاء والهيموجلوبين في الدم والصفائح الدموية. أظهرت نتائج الحقن بعقار السيكلوسبورين انخفاضاً كبيراً في الجلوبيولين المناعي في الدم والأجسام المناعية بالإضافة إلى زيادة المانداليد في أنسجة الطحال. المكمل بمسحوق بذور الأفوكادو مع السيكلوسبورين ، وتحسين نشاط الإنزيمات المضادة للأكسدة في أنسجة الطحال والجلوبيولين المناعي في الدم. أشارت النتيجة إلى أن بذور الأفوكادو حسنت جهاز المناعة في الفئران التي حقنت بعقار السيكلوسبورين المثبط للمناعة.