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Modulatory Effects of Vitamin C in Innate and Adaptive Immune Responses in Male Albino Rats

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ABSTRACT

This study was designed to assess the effectiveness of vitamin C in ameliorating the alterations in innate, adaptive, humoral and cell-mediated immune responses induced by luminal administration in rats. Eighty-four male albino rats (160-180 g) were divided into four equal groups. The first, the control group, was injected daily and intraperitoneally (i.p) with saline solution (0.9 % NaCl) for 21 days. The second group was given a daily i.p injection of luminal (50mg/kg). The third group was orally and daily administered with vitamin C (50mg/kg). The last group was treated the same way as the second group plus an oral administration of VC (50mg/kg) after half an hour of luminal injection. Determination of the alterations in different immunological parameters took place after the first, the second and third weeks of luminal and vitamin C administration. It was found that administration of luminal increased significantly the total leucocyte count, the percentage of lymphocytes and the chemokinetic index of leukocytes. Also, the concentration of total immunoglobulin increased significantly at the second and third weeks of luminal intake. While, marked decrease was noticed in the neutrophils percentage, phagocytic activity of polymorphonuclear leukocytes in vitro and immunoglobulin level at the first week of luminal intake. The chemotaxis activity of leucocytes towards luminal showed moderate response in all first week treated subgroups. The precipitation of antibodies against luminal antigen took place. The migration inhibition factor of leukocytes showed a marked decrease as a result of luminal intake at the third week. No significant changes were noticed in the current study concerning the percentages of monocytes, acidophils and basophils. Administration of vitamin C alone induced a mild increase in total leukocytes count, monocytes, neutrophils and basophils percentages and significant increase in phagocytic activity of leukocytes in vitro. On the other hand, the combined administration of vitamin C with luminal ameliorated some immunological alterations induced by luminal injection. Vitamin C apparently alleviated the total leukocyte count, the percentage of neutrophils, the values of phagocytic activity of polymorphonuclear leukocytes in vitro and total immunoglobulin concentration compared to the corresponding control subgroups. The chemotaxis activity showed moderate response in the third week of luminal & vitamin C subgroup. In all luminal & vitamin C subgroups, precipitation of antibodies against luminal antigen was recorded. At the third week, all luminal, vitamin C and luminal & vitamin C subgroups revealed significant decrease in the migration inhibition factor of leukocytes versus the control subgroups. Conclusion: the current study revealed that administration of vitamin C along with luminal exerted immunomodulatory effects against luminal-induced alterations in the immune system and that it could partially affect innate, adaptive, humoral and cell mediated immune responses in male albino rats.

Keywords: Immune system, Vitamin C, Luminal, Phagocytic activity, Chemotaxis, Albino rats

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1. Introduction

The immune system can be divided into epithelial barriers, and cellular and humoral constituents. Beyond to structural and chemical barriers to pathogens, the immune system has two fundamental lines of defense: innate immunity and adaptive immunity. Innate immunity is the first immunological mechanism for fighting against an intruding pathogen. It is a rapid immune response, and has no immunologic memory. Adaptive immunity, on the other hand, is antigen-dependent and antigen-specific; it has the capacity for memory (Bonilla and Oettgen, 2010). There is a great deal of synergy between the adaptive immune system and its innate counterpart, and defects in either system can provoke illness or disease, such as inappropriate inflammation, autoimmune diseases, immunodeficiency disorders and hypersensitivity reactions (Marshall *et al.,* 2018). The role of nutrition in immunity has received increased attention in the last few decades. A lot of interest has been given to the effect of essential nutrients such as vitamins on specific aspects of immune function (Maggini *et al.,* 2017).

Vitamin C, also known as ascorbic acid, is a well-known antioxidant that can act as a cofactor for several enzymes involved in biosynthesis and regulation of gene expression. Humans have an absolute requirement for vitamin C as a part of their diet and deficiency due to inadequate intake is associated with a plethora of symptoms, reflecting the diverse functions attributed to the vitamin (Banhegyi *et al.,* 2014). Numerous studies have demonstrated that vitamin C supplementation enhances the immune cells response, influences the outcome and progression of diabetic complications, prevents DNA damage, and significantly decreases the risk of a wide range of pathologies such as cancers and degenerative and chronic diseases. In humans, severe vitamin C deficiency has been associated with impairments in immunity and increased susceptibility to more infections, while vitamin C supplementation seems helpful to prevent and treat infections (Webb and Villamor, 2007 and Hemila, 2017).

Luminal (phenobarbital), one of the more commonly used sedatives, is used alone or with other medications to control and reduce seizures, so, leads to more normal daily activities, reduces risk of harm on losing consciousness, and lessens risk for a possibly life-threatening condition of frequent, repeated seizures. Luminal belongs to a class of drugs known as barbiturate and works by controlling the abnormal electrical activity in the brain that occurs during a seizure. This medication is also used for a short time to help for calm or sleep during periods of anxiety. It works by affecting certain parts of the brain to cause calming (Falco and Bleck, 2016 and Nakayama *et al.*, 2019).

Increasing body of evidence indicates that besides the central nervous system, antiepileptic drugs may also affect the function of the immune system. Experimental data showed that classical antiepileptic drugs affect peripheral immunological parameters and attenuate both humoral and cellular responses, and an engagement of CD8+ cells in these effects was suggested. Other authors reported that luminal, behaves as an immunosuppressant *in vivo* and *in vitro* when prescribed in conventional, clinically acceptable doses. In addition, Previous studies indicated that luminal induce hypersensitivity of immune system and diminish humoral response and lymphocyte T cytotoxicity in mice (Vezzani *et al.,* 2012).

Supplementation of vitamin C to some animal species has produced conflicting results. Vitamin C deficiency is linked to compromised immunocompetence and elevated ascorbic acid concentration is linked to increased immune responses in catfish, poultry, cattle, and swine. Conversely, vitamin C supplementation has had no beneficial effects on the immune functions measured in mice, guinea pigs, and cattle (Eicher *et al.,* 1992)

The present work aimed to evaluate the protective and immunomodulatory effects of vitamin C against disturbances in innate, adaptive, cell-mediated and humoral immune responses induced by luminal intake in adult male albino rats.

2. Materials and Methods

2.1. Drugs

Luminal and vitamin C tablets were purchased from a pharmacy of Minia university hospital. Luminal was prepared as phenobarbital sodium (Merk) dissolved in sterile physiological saline

solution (0.9% NaCL) in a dose of (50 ml/Kg body weight) (Abdel-Raheem *et al.,* 2002). Luminal dose was given by intraperitoneal injection. Vitamin C was given in a dose of (50 ml/Kg body weight). It was dissolved in distilled water and was administered orally by oral administration tube. All the other chemical reagents and kits used in the study were of standard analytical grades and purchased from Sigma Chemical Co. (St. Louis, O, USA).

2.2. Animals groups and study design

Eighty- four male albino rats (body weight, 160-180 g) were used in the present study. They were kept in cages, fed on commercial food and water was supplied. They were divided into four groups, each of twenty-one animals. The first is the control group that was subdivided into three subgroups each of seven animals; first, second and third week subgroups. All subgroups were daily intraperitoneally injected with saline solution (0.9% NaCl) (1m/g body weight). They were sacrificed on the seventh, fourteenth and the twenty-first days post-injection. The second group is luminal group which was daily intraperitoneally injected with luminal (50mg/kg body weight) as Phenobarbital sodium (Merk) dissolved in 0.9% NaCl. They were subdivided into three subgroups each of seven animals; first, second and third week subgroups. They were sacrificed on the seventh, fourteenth and twenty-first days post-injection. The third is vitamin C (VC) group that was daily and orally administered by oral administration tube with vitamin C (50mg/kg body weight) dissolved in distilled water. They were subdivided into three subgroups each of seven animals; first, second and third week subgroups. They were sacrificed on the seventh, fourteenth and twenty- first days post- administration. While, the fourth is luminal & vitamin C group (luminal/VC) that was daily treated by luminal in the same way as luminal group, then was given vitamin C by oral administration like those of vitamin C group after half an hour of luminal injection. They were subdivided into three subgroups each of seven animals; first, second and third week subgroups. They were sacrificed on the seventh, fourteenth and twenty- first days post- injection and post-administration.

At the end of each experimental period in different subgroups, animals were fasted overnight but allowed free access to water. Animals were sacrificed under anesthesia with diethyl ether. Blood samples were collected from each sacrificed animal into two clean centrifuge tubes (one heparinized and the other is non – heparinized). The non –heparinized blood was centrifuged at 3000 r.p.m for 15 minutes to get serum, which kept at- 80^{o}C.

2.3. Methods

2.3.1. Leukocytes:

2.3.1.1. Determination of Total Leukocyte counts

Direct counting of white blood cells (W.B.Cs) on the haemocytometer counting chamber was used, using Turk's solution, which consists of 1ml glacial acetic acid, 1ml of aqueous 1% gentian violet solution, and 100 ml distilled water.

2.3.1.2. Determination of Differential Leukocyte Counts

It was carried out by staining thin blood films with Giemsa stain, which was examined microscopically using oil immersion. Percentage of each type of leucocytes in relation to the total number of leukocyte count was calculated.

2.4. Innate Immunity:

The innate immunity response to luminal was determined using the techniques of phagocytosis *in vitro* and chemotaxis and chemokinasis assays

2.4.1. Phagocytosis *in vitro*

The phagocytosis *in vitro* of polymorphonuclear leukocytes using Candida albicans was established by separation of polymorphonuclear cells using Ficoll-histopaque-1119 and Ficoll-histopaque-1077 (Sigma-diagnostics) as described by Wilkinson (1981).

2.4.2. Chemotaxis and Chemokinesis Assays

The two assays system was used to study the migration of leukocytes in active and directional events.

2.4.2.1. The agarose gel assay (chemotaxis movement assay):

The chemotaxis assay was used to study the chemotactic response of leukocytes in response to luminal. The method was described by Nelson *et al*. (1975) and modified by Gearing & Rimmer. (1985).

2.4.2.2. The micropore filter assay (chemokinetic assay)

The chemokinetic assay was established according to the method of Gearing & Rimmer. (1985) and El- Feki. (1994).

2.5. Humoral Immunity (B-cells):

The humoral response to luminal was determined using the following techniques, detection of precipitation antibodies, and quantitative determination of total immunoglobulin by zinc sulfate turbidity.

2.5.1. Detection of antibodies precipitation

Precipitation of antibodies was determined by a method described by El- Feki *et al.* (1998).

2.5.2. Quantitative Determination of Total Immunoglobulin by Zinc Sulfate Turbidity

A laboratory method for quantitative determination of serum immunoglobulin by zinc sulfate turbidity described by Pfeiffer *et al*. (1977) was used. This method is depended on the biochemical properties of immunoglobulin, in relation to characteristic of Zn SO_4.

2.6. Cell Mediated Immunity (T-cells):

2.6.1. Inhibition of Leukocyte Migration Capillary Technique (MIF)

Inhibition of Leukocyte migration capillary technique was used to determine migration inhibition factor of leukocyte (MIF) according to the methods of (Abu El-Saad, 1996 and El-Feki *et al*., 1998).

2.7. Statistical Analysis:

The data of all groups were represented in tables as mean ± standard deviation (SD) and were statistically analyzed by multi-factor analysis of variance (MANOVA test). The accepted level of significance between all treated groups was $P<0.05$, $P<0.01$ and $P<0.001$.

3. Results

3.1. Leukocytes Determinations:

3.1.1. Total Leukocyte Counts:

Estimation of the effect of Luminal, vitamin C and Luminal & vitamin C on the total leukocyte counts (Table 1) showed a marked increase in Luminal and luminal & vitamin C groups. Luminal treatment induced a significant increase at $P<0.05$ at the second week in comparison to control subgroup, while, first and third week Luminal subgroups revealed non-significant increase.

Table 1: Effects of luminal and/or vitamin C (VC) on the total leukocyte count ($\times 10^3$).

Treatment Groups	Control	Luminal	VC	Luminal/VC
1st w. Group	9.45 ± 0.75	9.91± 0.66	9.63 ± 0.63	9.17 ± 0.98
2nd w. Group	9.38 ± 0.81	11.09 ± 1.07 *	9.54 ± 0.65	9.12 ± 0.73
3rd w. Group	9.48 ± 0.52	10.25 ± 0.69	9.47 ± 0.56	10.1 ± 0.89 *

Significant increase or decrease compared with the control group (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).
Data are expressed as mean ± S.D (n = 7).

On the other hand, mild and non-significant increase was recorded by vitamin C treatment in the first and second week subgroups. Luminal & vitamin C treatment induced a significant increase at the third week versus the control subgroup, while, the first and second week subgroups revealed non- significant decrease compared with corresponding controls.

3.1.2. Differential Count of Leukocytes:

Collected data from the effect of Luminal, vitamin C and Luminal & vitamin C on the differential count of leukocytes are given in table (2). Lymphocyte percentages were increased by the effect of both Luminal and Luminal & vitamin C administration, where first week Luminal and Luminal & vitamin C subgroups revealed significant increases (P<0.05) in the lymphocytes percentage versus the control one, while second and third week subgroups revealed non-significant increases in lymphocyte percentages. No significant change in the lymphocyte percentages was induced by vitamin C treatment in all periods of treatment.

Table 2: Effects of luminal and/or vitamin C (VC) on the lymphocyte differential count (%).

Treatment Groups	Control	Luminal	VC	Luminal/VC
1st w. Group	73.66 ± 3.57	79.62 ± 2.23 *	73.51 ± 4.08	78.44 ± 5.25 *
2nd w. Group	76.95 ± 5.35	79.37 ± 5.10	75.60 ± 4.63	79.39 ± 4.58
3rd w. Group	74.38 ± 4.25	76.92 ± 4.48	74.04 ± 4.51	78.53 ± 3.72

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

Concerning monocyte percentages, there was no significant changes in all studied subgroups comparing with the corresponding controls. While, a mild increase was noticed at the first and second week of vitamin C subgroups (Table 3).

Table 3: Effects of luminal and/or vitamin C (VC) on the monocytes differential count (%).

Treatment Groups	Control	Luminal	VC	PB/VC
1st w. Group	3.93 ± 0.82	3.99 ± 0.53	4.14 ± 0.52	3.86 ± 0.\64
2nd w. Group	3.95 ± 0.59	3.90 ± 0.40	4.31 ± 0.45	4.01 ± 0.38
3rd w. Group	3.97 ± 0.57	4.04 ± 0.54	3.97 ± 0.53	4.03 ± 0.44

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

Luminal and Luminal & vitamin C treatment decreased the neutrophils percentage, where first week Luminal and Luminal & vitamin C subgroups revealed significant decrease at P<0.05 in the neutrophils percentage compared with the control subgroup, while, second and third week subgroups revealed non-significant decreases in the neutrophils percentage compared with the corresponding controls. Vitamin C treatment induced non-significant increases in the neutrophils percentage in the second and third week subgroups versus the corresponding controls (Table 4).

Table 4: Effects of luminal and/or vitamin C (VC) on the neutrophils differential count (%).

Treatment Groups	Control	Luminal	VC	Luminal/VC
1st w. Group	19.93 ± 4.01	13.83 ± 1.60 *	19.40 ± 3.77	15.32 ± 4.93 *
2nd w. Group	16.40 ± 5.12	13.92 ± 5.01	17.24 ± 4.20	14.59 ± 4.75
3rd w. Group	18.88 ± 4.40	15.89 ± 4.64	19.23 ± 4.73	16.82 ± 3.43

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

Concerning the acidophils percentage, no significant changes were noted, while luminal subgroups revealed a non-significant increase in the acidophils percentage versus the control subgroups, (Table 5).

Table 5: Effects of luminal and/or vitamin C (VC) on the acidophils differential count (%).

Treatment Groups	Control	Luminal	VC	Luminal/VC
1st w. Group	1.93 ± 0.32	2.05 ± 0.26	2.07 ± 0.53	2.03 ± 0.30
2nd w. Group	2.04 ± 0.24	2.22 ± 0.32	2.09 ± 0.29	2.02 ± 0.22
3rd w. Group	1.99 ± 0.26	2.21 ± 0.31	2.09 ± 0.26	2.08 ± 0.38

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

Also, the basophils percentage did not significantly change in all studied subgroups, but, a mild decrease was noticed in luminal subgroups compared with the corresponding controls. On the other hand, vitamin C subgroups showed a mild increase in basophils percentage compared with the corresponding controls at the first and second week subgroups (Table 6).

Table 6: Effects of luminal and/or vitamin C (VC) on the basophils differential count (%).

Treatment Groups	Control	Luminal	VC	Luminal/VC
1st w. Group	0.66 ± 0.33	0.58 ± 0.39	0.79 ±0.19	0.65 ± 0.28
2nd w. Group	0.65 ± 0.31	0.63 ± 0.28	0.70 ± 0.30	0.76 ± 0.16
3rd w. Group	0.77 ± 0.21	0.64 ± 0.30	0.66 ± 0.34	0.65 ± 0.29

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

3.2. Innate Immunity:

3.2.1. Phagocytosis *in vitro:*

The effect of Luminal, vitamin C and Luminal & vitamin C on polymorphonuclear leukocyte phagocytic activity *in vitro* is represented in tables (7). Estimation of phagocytic activity showed a marked decline in luminal subgroups versus the controls. This decline was of a significant value (P<0.05) at the first week subgroup, while, second and third week luminal subgroups revealed non-significant decreases. Vitamin C subgroups showed non-significant increase in comparison to control subgroups. This increase was of a significant value (P<0.05) at the first week subgroup. The combined administration of vitamin C with Luminal induced significant increase (P<0.05) at the first week subgroup compared with the corresponding control.

Table 7: Effects of luminal and/or vitamin C (VC) on the phagocytic activity of polymorphonuclear leukocytes *in vitro* (%).

Treatment Groups	Control	Luminal	VC	Luminal/VC
1st w. Group	33.15 ± 3.67	28.96 ± 2.57 *	36.96 ± 4.03 *	34.40 ± 2.90 *
2nd w. Group	28.19 ± 4.30	24.90 ± 4.06	31.17 ± 4.17	26.06 ± 3.19
3rd w. Group	32.36 ± 4.75	30.77 ± 2.93	33.86 ± 4.70	31.10 ± 3.99

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

3.2.2. Chemotaxis and Chemokinesis:

Data recorded from the effect of Luminal, vitamin C or Luminal & vitamin C on the chemotaxis activity of leukocytes towards Luminal (Table 8) showed moderate response of leucocytes to Luminal in all first week subgroups, while in the second and third week of treatment, no changes were recorded except the moderate response in the third week luminal & vitamin C subgroup.

Estimation of the chemokinetic index of blood leukocytes (Table 9) showed significant increase (P<0.05) in the first and second week Luminal subgroups, while, a non-significant increase was recorded at the third week. No significant changes were noted in the vitamin C subgroups. Also, non-significant increase in the chemokinetic index was recorded at the second and third week Luminal & vitamin C subgroups.

Table 8: Effects of luminal and/or vitamin C (VC) on leukocytes chemotaxis activity.

Groups	Control		Luminal		VC		Luminal/VC	
	C	T	C	T	C	T	C	T
1st. Week	++	+++	+	++	++	+++	++	+++
2nd. Week	++	++	+	+	++	++	++	++
3rd. Week	++	++	++	++	+++	+++	++	+++

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

\- No taxis were recorded + Faint ++ Moderate +++ High +++ Strong

Table 9: Effects of luminal and/or vitamin C (VC) on leukocytes chemokinetic index.

Treatment Groups	Control	Luminal	VC	Luminal/VC
1st w. Group	1.05 ± 0.06	1.18 ± 0.12 *	1.05 ± 0.10	1.05 ± 0.09
2nd w. Group	1.00 ± 0.07	1.13 ± 0.09 *	1.03 ± 0.08	1.09 ± 0.07
3rd w. Group	0.93 ± 0.08	1.01 ± 0.13	1.03 ± 0.07	1.07 ± 0.10

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

3.3. Humoral Immunity:

3.3.1. Precipitation of Antibodies:

Estimation of the effect of Luminal, vitamin C and Luminal & vitamin C on the precipitation of antibodies against Luminal antigen (Table 10) showed that no precipitation took place in both control and vitamin C subgroups. On the other hand, in luminal and luminal& vitamin C subgroups precipitation took place. In Luminal subgroups, precipitation took place at the second and third week luminal subgroups, while, no precipitation was found in the first week subgroup. Furthermore, in all Luminal & vitamin C subgroups precipitation was recorded.

Table 10: Effects of luminal and/or vitamin C (VC) on the precipitation of antibodies

Groups		Time		
		1st. Week	**2nd. Week**	**3rd Week**
	Control	-	-	-
	Luminal	-	+	+
	VC	-	-	-
	Luminal/VC	+	+	+

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

\- No precipitation + Positive precipitation

3.3.2. Total Immunoglobulin in Serum:

Data recorded from the effect of Luminal, vitamin C and Luminal & vitamin C on the total immunoglobulin concentrations in serum are represented in tables (11). Luminal treatment induced a significant decrease (P<0.05) in the first week Luminal subgroup. On the other hand, the concentration of total immunoglobulin increased significantly (P<0.05) at the second week and non-significantly at the third week. No significant effect was induced by vitamin C subgroups. Luminal & vitamin C subgroups revealed significant increase (P<0.05) at the third week, while the first and second weeks subgroups revealed non-significant changes.

3.4. Cell-Mediated Immunity:

3.4.1. Migration Inhibition Factor:

Estimation of the migration inhibition factor (MIF) of leukocytes is demonstrated in table (12).

At the first and second weeks, both Luminal and Luminal & vitamin C subgroups revealed non-significant decreases versus the control subgroups, while vitamin C subgroups had slight

decrease than the control subgroups. At the third week, all Luminal, vitamin C and Luminal & vitamin C subgroups revealed significant decrease at P<0.05 versus the control subgroup.

Table 11: Effects of luminal and/or vitamin C (VC) on serum total immunoglobulin concentration (mg %).

Treatment Groups	Control	Luminal	VC	Luminal/VC
1st w. Group	2072.83 ± 118.47	1787.83 ± 203.95*	1978.00 ± 164.08	2058.00 ± 196.00
2nd w. Group	2070.00 ± 183.21	2338.00 ± 132.86*	2002.00 ± 173.38	2088.67 ± 263.12
3rd w. Group	1831.33 ± 101.45	1871.50 ± 159.81	1712.67 ± 47.36	2153.17 ± 132.75 *

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

Table 12: Effects of luminal and/or vitamin C (VC) on leukocyte migration inhibition factor (MIF).

Treatment Groups	Control	Luminal	VC	Luminal/VC
1st w. Group	0.53 ± 0.09	0.45 ± 0.14	0.50 ± 0.08	0.46 ± 0.12
2nd w. Group	0.59 ± 0.13	0.47 ± 0.12	0.57 ± 0.13	0.41 ± 0.09
3rd w. Group	0.60 ± 0.06	0.47 ± 0.09 *	0.51 ± 0.07 *	0.46 ± 0.07 *

Significant increase or decrease compared with the control group (* P<0.05, ** P<0.01, *** P<0.001).
Data are expressed as mean ± S.D (n = 7).

4. Discussion

Since its discovery more than 80 years ago, the function of vitamin C in the immune system has been the subject of much research and more than a little controversy. One of the main drivers of this interest is that leukocytes accumulate the vitamin to high intracellular concentrations, so, signalling an important role for it in these cells (Levine *et al.,* 2001). Intracellular levels generally respond to variations in plasma ascorbate availability, and a combination of inadequate intake and increased turnover during severe stress can result in low plasma ascorbate status. Intracellular ascorbate supports essential functions and, in particular, acts as an enzyme cofactor for Fe- or Cu-containing oxygenases, newly discovered enzymes in this family, that regulate cell metabolism and epigenetics, and dysregulation of their activity can affect cell phenotype, growth and survival pathways, and stem cell phenotype (Abel *et al.,* 2018).

In the current study, the total leukocyte count was increased after administration of luminal that can be considered as an inflammatory response induced as a defense mechanism against the injected antigen (EL-Shahawy *et al.,* 1995 and Anitra and Silvia, 2017). Luminal treatment induced the inflammatory process, which could be mediated by stimulated macrophages. Macrophages form the following factors as a response of inflammation, tumor necrosis factor (TNF), interleukin-1 (IL-1), granulocyte-monocyte colony-stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), and monocyte colony stimulating factor (M-CSF). The GM-CSF stimulates both granulocytes and monocytes production by the bone marrow. The combination of TNF, IL-1 and colony stimulating factors provides a powerful feedback mechanism that begins with tissue inflammation and then induces the formation of defensive white blood cells, which increase leukocyte count (Levy *et al.,* 1991 and Murphy *et al.,* 2007).

The combined administration of vitamin C with luminal reduced the increase induced by luminal in the total leukocyte count at the first and second weeks of treatment. This may be due to the protective effect of vitamin C against the inflammation induced by luminal, where, the extracellular vitamin C may serve as a physiological protecting agent against oxygen radical damage in inflammation as polymorphonuclear leukocytes are known to produce superoxide and oxygen derivatives upon activation (Mikirova *et al.,* 2013).

Studied blood films in the present study indicated an increase in the lymphocytes percentage after the treatment with luminal or luminal& vitamin C which may be due to the chronic

inflammation induced by luminal. Lymphocytes play the key role in all immune responses, and their activity is always directed against specific foreign agents (wheater *et al.,* 1979). The acquired immunity against luminal activates both B and T lymphocytes, where, antigens bind to the antibodies on the B cells surface. Antigen-antibody binding activates the B cell and triggers rapid series of mitotic divisions. Also, antigen binds to T cell receptors, causing it to divide repeatedly to form a clone of identical sensitized T cells. Also, Gonzalez *et al.* (1997) mentioned that the allergic effect of luminal involves the activation of T cell proliferation. The role of vitamin C in lymphocytes is less clear, but it has been shown to enhance differentiation and proliferation of B- and T-cells, likely due to its gene regulating effects (Anitra and Silvia, 2017).

The kinetics of the immune response of the treated animals was described via the innate, humoral and cell-mediated responses. The first and often most important response to infectious agents is the non-specific immunity. Numerous cells are involved in the innate immune response such as phagocytes (macrophages and neutrophils), dendritic cells, mast cells, basophils, eosinophils, natural killer (NK) cells and innate lymphoid cells (Marshall *et al.,* 2018). Neutrophils are short-lived cells that are the first responders to an inflammatory challenge. Their recruitment to, and clearance from, inflammatory sites is dependent on the regulation of cell death and survival pathways (Maianski *et al.,* 2004). In the current study, treatment with Luminal and Luminal & vitamin C decreased the neutrophils percentage that may be due to the migration of neutrophils to the inflamed tissues. Froscher & Kleinhans (1998) demonstrated allergic effect of Luminal and activated acidophils released histamine, which increase the attraction of neutrophils to the active sites. Vitamin C is thought to influence several important aspects of neutrophil function: migration in response to inflammatory mediators (chemotaxis), phagocytosis and killing of microbes, and apoptosis and clearance by macrophages (Anitra and Silvia, 2017). The insignificant increase in the acidophils percentage after Luminal treatment may be due to the activation of acidophils as allergic response to Luminal (Gonzalez *et al.,* 1997). The combined administration of vitamin C with Luminal reduced this increase that may be as a result of the effect of vitamin C in the treatment of allergy (Kodama *et al.,* 1994).

Phagocytosis is the principle mechanism for destruction of extracellular pathogens, as well as, several viral and fungal organisms in mammals. The first encounter of the host with a foreign organism leads to a serotype response consisting of mobilization of phagocytic elements into areas where foreign organism has invaded. Once mobilized, the phagocytic cells mount an attack on the target by proceeding through a series of associated steps (Turvey and Broide, 2010). Phagocytes are sub-divided into two main cell types: neutrophils and macrophages. In addition to their phagocytic properties, neutrophils contain granules and enzyme pathways that assist in the elimination of pathogens (Marshall *et al.,* 2018). In the present study, the phagocytic activity of the separated leukocytes was determined *in vitro* against candida albicanas. Treatments with Luminal decreased the phagocytic activity of polymorphonuclear leukocytes. This decrease may be related to the immunosuppressive effect exhibited by Luminal (Marcoli *et al.,* 1985) or may be related to the decrease in neutrophils induced by Luminal as recorded from studied blood films. Also, the decrease in the phagocytic activity may be attributed to the increased migration inhibition factor (MIF) activity induced by Luminal, which suppressed the movement and the mobility of macrophages and neutrophils to reach the inflamed areas (El-Feki *et al.,* 1995). Because of the structural similarity between glucose and vitamin C, the accumulation of vitamin C in neutrophils can be regulated by glucose (Washko &Levine, 1992). The site of vitamin C transport across the membranes is probably the same as of glucose (Moser & Weber, 1984). The absence of insulin, the glucose regulating hormone, significantly reduced vitamin C uptake by cells (Kapeghian & Verlangienri, 1984). The decrease in insulin secretion induced by Luminal (Jones *et al.,* 1994) may explain the decrease in the phagocytic activity induced by luminal as a result of decrease vitamin C uptake by polymorphonuclear leukocytes.

The increase in the phagocytic activity after vitamin C treatment may be attributed to the stimulatory effect of vitamin C on the phagocytic cells as vitamin C is maintained at high levels in most immune cells and can affect many aspects of the immune response. Previous studies

demonstrated that vitamin C plays an important role in the phagocytic activity of leukocytes, where high dietary dose of vitamin C induced phagocytic activation of leukocytes when the cells were stimulated with substrate (Hernanz *et al.,* 1990, Dunier *et al.,* 1995 and Verlhac *et al.,* 1996). Also, adherent kidney and spleen cells phagocytic capacity in the turbot showed a positive correlation with dietary vitamin C levels (Roberts *et al.,* 1995). The phagocytic cells, neutrophils and macrophages, contain 10-40 times increased intracellular vitamin C concentration compared to plasma (Oberriter *et al.,* 1986).

The role of vitamin C in activation of polymorphonuclear leukocyte phagocytic cells like neutrophils has been investigated thoroughly and was recently reviewed (Carr and Maggini, 2017). This essential role is attributed to that vitamin C acts as a scavenger of oxidant derived from polymorphonuclear leukocytes. Because of vitamin C is the major antioxidant, therefore, it may be an important physiologic factor in counteracting the oxidants released by neutrophils as activation of neutrophils *in vitro* causes rapid oxidation of extracellular vitamin C (Hemilae,1992). The combined administration of vitamin C with Luminal in the current study ameliorated the decrease induced by Luminal in the phagocytic activity especially at the first week of treatment, which may be related to the anti-immunosuppressive effect of vitamin C (Carr and Maggini, 2017). Also, Heuser & Vojdani (1997) mentioned that, the immune functional abnormalities after toxic chemical exposure, could be restored by the oral usage of vitamin C.

Cell migration is often seen in response to the presence of chemical substances in the environment by immunocompetent cells (leukocytes) that migrate to and become localized at sites of inflammation. Inflammation is a cellular response of the immune system to microbial invasion and / or tissue injury. Chemotaxis and subsequent phagocytosis are two integral components of the inflammatory response and innate immunity. Chemotactic migration of leukocytes to the site of inflammation is an active and directional event, the leukocytes move from the blood to the inflamed tissue along chemical gradients originating at the site of inflammation (Obenauf & Smith, 1985). The factors stimulating this migration may be of microbial or host origin. When leukocyte migration is directional along an increasing concentration gradient of an attracting substance, it is termed chemotaxis, while, if the response to the substance is of increased speed or frequency of migration, it is termed chemokinesis (Marshall *et al.,* 2018).

Chemotaxis studied in the present work showed a moderate increase in the directional movement of leukocytes to Luminal chemoattractant at the first week group and also at the third week Luminal & vitamin C subgroup, while the chemokinetic response of leukocytes revealed a marked increase in the Luminal subgroups. Increased chemotaxis activity of leukocytes by Luminal was recorded previously by Laskin *et al.* (1988). The increased chemotaxis activity at the first and third week Luminal & vitamin C subgroup may be related to the effect of vitamin C on the enhancement of leukocyte chemotactic activity as mentioned by Nowaket *et al.* (1989). The inflammation induced by Luminal (Levy *et al.,* 1991) include stimulation of leukocytes, which release cytokines including interleukin-1 alpha (IL-1 alpha), interleukin-1 beta (IL-1 beta), interleukin-2 (IL-2), and interleukin-6 (Pacifici *et al*., 1995) and some of these cytokines (IL-1) are chemotactic factors. Otherwise, Luminal induces histamine release from mast cells (Masini *et al*., 1990 and Marshall *et al.,* 2018) that activates the chemotactic activity of leukocytes. The combined administration of vitamin C with Luminal restored the alteration induced by Luminal in the chemokinetic index that may be due to the effect of vitamin C in the treatment of allergic effect of Luminal (Kodama *et al.,* 1994 and Anitra and Silvia, 2017).

B lymphocytes are at the center of the adaptive, humoral immune system. They are responsible for the production of antigen-specific immunoglobulin (Ig) directed against invasive pathogens (antibodies). Similar to other leukocytes, vitamin C accumulates in B lymphocytes but there is only limited data on the function of vitamin C in these cells (Gwendolyn *et al.,* 2018).

Concerning the detection of precipitation of antibodies against Luminal antigen in the current study, an immunoprecipitation lines were noted in both Luminal and Luminal & vitamin C groups that indicated the formation of specific antibodies against Luminal in these groups. Preparation of monoclonal anti-luminal antibodies has been demonstrated by Danilova *et al*.

(1995). The crystallographic structure of this anti-luminal antibody indicated that it is from IgG1 type (Harris *et al*., 1998). Otherwise, Luminal conjugation with albumin plasma protein acquires an ability to induce the immunological system (Archakov *et al.,* 1980) that leads to appearance of antibodies specifically precipitate with Luminal (Kovalev *et al.,* 1975). On the other hand, the quantitative determination of total immunoglobulin in the present study showed a decrease at the first week Luminal subgroup, while continuous treatment with Luminal induced a marked increase at the second and third weeks. Also, combined administration of vitamin C with Luminal caused increase in the total immunoglobulin at the third week. The decrease induced at the first week by Luminal treatment may be due to the immunosuppressant effect of Luminal in lymphocyte subpopulation as mentioned by Yang-Kuender *et al.* (1992). The combined administration of vitamin C with Luminal at the first week ameliorated the level of total immunoglobulin that may be related to the effect of vitamin C in the improvement of immune-functional abnormalities (Heuser & Vojdani, 1997) and increasing the antibodies levels (Anitra and Silvia, 2017). The increase in the total immunoglobulin at the second week after Luminal treatment may be related to the formation of anti-luminal antibodies as detected from the previous precipitation technique. Also, this increase in the immunoglobulin may be related to the formation of autoantibodies against cytochrome P450 enzymes induced by Luminal as mentioned by Riley *et al.,* 1993. The increase in total immunoglobulin induced by the combined administration of vitamin C with Luminal at the third week of treatment may be due to the stimulating effect of vitamin C which increases the humoral response against Luminal (Bergsten *et al.*, 1990 and Dunier *et al.,* 1995). This activation also may be as a result of rapid accumulation of vitamin C inside the mononuclear leukocytes (Ichiyama *et al.,* 2009). It is possible that vitamin C has an effect on the proliferation and function of B lymphocytes and antibodies production but the results until now are inconclusive (Gwendolyn *et al.,* 2018). A previous study investigated the effect of vitamin C on immune responses in human peripheral blood lymphocytes cultured for 7 days with and without vitamin C before stimulating them with pokeweed mitogen (PWM), a T cell dependent B cell stimulus. The cultures treated with vitamin C showed an increased number of IgM and IgG-secreting cells after stimulation and the increase was dose-dependent (Gwendolyn *et al.,* 2018).

The cell-mediated response against Luminal was established by the measurement of the migration inhibition factor (MIF). Both Luminal and Luminal & vitamin C subgroups revealed a decrease in the MIF after treatment. This decrease was in accordance with the results of Howell (1987) who showed that following *in vivo* sensitization with antigen; the migration of leukocytes was reduced after *in vitro* challenge with the specific sensitizing antigen. knutsen *et al*. (1984) mentioned that *in vitro* lymphocytes stimulation with luminal showed a cell-mediated hypersensitivity reaction to luminal. The activation of T cells in the allergic effect of Luminal was recorded by Gonzalez *et al.* (1997) that may be a result of the presence of Luminal receptors on the lymphocytes as mentioned by Ferrarese *et al.* (1995) who demonstrated Luminal receptors in human lymphocytes. These receptors may be used as peripheral markers of anticonvulsant drug effect that increased after Luminal treatment which caused immunological alterations. This activated T cell by Luminal released MIF, which inhibits the migration of macrophages. The decrease in MIF after combined administration of vitamin C with Luminal may be due to the stimulatory effect of vitamin C on the development of the cell mediated immunity. Gwendolyn *et al.,* (2018) mentioned that, the established association of vitamin C with modulation of the immune response included activation of T cell- mediated immunity.

5. Conclusion:

Vitamin C is an essential micronutrient for humans, with pleiotropic functions related to its potent antioxidant effect and its ability to ameliorate various cellular functions of both the innate and adaptive immune system. The current study revealed that administration of vitamin C along with luminal exerted immunomodulatory effects against luminal-induced alterations in the immune system and that it could partially affect innate, adaptive, humoral and cell mediated immune responses in male albino rats.

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