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SOME IMMUNOMODULATING EFFECTS OF ATORVASTATIN

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ABSTRACT:

The present study was conducted to investigate the immune-pharmacological effects of atorvastatin on the cellular and humoral immune response of rabbits (either non-vaccinated or vaccinated with rabbit hemorrhagic viral disease vaccine). Two blood samples were collected from each rabbit (5rabbits /group) at the 1st and 3rd day, 1st, 2nd and 3rd week post vaccination and/or drug administration for studying both cellular and humoral immune response.

Key Words: Cancer, Tumors, Pain, Nausea and Vomiting, Atorvastatin, Cellular and Humeral Immunity.

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INTRODUCTION:

Statins are compounds of natural origin that are biosynthesized as secondary metabolites of several filamentous fungi and act as competitive inhibitors of HMG-CoA reductase[1]. Atorvastatin is a synthetic lipid-lowering agent, which is an inhibitor of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis that converts 3-hydroxy-3-methyl-glutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol. Triglycerides (TG) and cholesterol in the liver are combined into very low-density lipoprotein (VLDL) and released into plasma to be delivered to peripheral tissues. Low-density lipoprotein (LDL) is framed from VLDL and is catabolised primarily through the high affinity LDL receptors[2]. Atorvastatin additionally decrease VLDL-C and TG and produce variable increase in HDL-C and apolipoproteinA-1(apo-1). This means that, atorvastatin reduces total-C, LDL-C, VLDL-C, apoB, TG and increase HDL-C in patient with isolated hypertriglyceridemia[3].

AIM OF THE WORK:

The main objective of this study is to investigate the immuno-modulatory effects of atorvastatin on cellular and humeral immune response of rabbits either non vaccinated or vaccinated with RHDV vaccine.

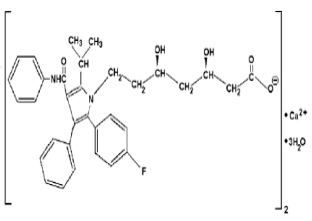
MATERIALS & METHODS:

A-MATERIALS:

I] Drug

Atorvastatin: It is used under trade name **Lipitor®.**

It is produced by **Pfizer Canada Inc.**



Dose:

The recommended therapeutic dose of atorvastatin for rabbit is 700µg/kg.b.wt[4].

II] Vaccine:

Inactivated rabbit hemorrhagic disease virus vaccine was used for active immunization of experimental rabbits. It was purchased from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt, and was given subcutaneously in a dose of 0.5 ml for each rabbit[5].

III] Rabbits:

A total of twenty (20) New Zealand white rabbits of 3-4 months old and weighing 2.5 kg were used in this work. They were divided into four equal groups each of five animals. Each group was kept in a separate cage in the battery. They were acclimatized for 2 weeks before beginning of the experiment in order to minimize possible stress effects and sure that all rabbits have been adapted to the same environmental conditions. Rabbits were fed on pelleted ration for rabbits twice daily from the day of arrival until the end of the experiment.

B-METHODS:

Experimental Design:

A total of twenty (20) New Zealand white rabbits of 3-4 months old and weighing about 2-2.5 kg were purchased from a private rabbitary without previous history of RHDV outbreaks or vaccination against RHDV, They were housed in disinfected metal cages in a well ventilated, well lightened and disinfected room. They received commercial pellet ration and clean water adlibitum, and kept under observation for 1 week before being used.

They were classified into 4 groups as the following:

1. **First group (G1):** was left as control, non-vaccinated non-treated group.
2. **Second group (G2):** Vaccinated, non-treated group was

Subcutaneously injected with inactivated rabbit hemorrhagic disease virus (RHVD) vaccine at dose of 0.5 ml per rabbit.

3. **Third group (G3):** Vaccinated-treated group was given single dose of atorvastatin 700µg/kg.b.wt orally, daily for one month then vaccinated by inactivated rabbit hemorrhagic disease virus (RHVD) vaccine was given 0.5ml/rabbit.
4. **Fourth group (G4):** Non-vaccinated, treated group was given a single dose of atorvastatin 700µg/ kg.b.wt orally, daily for one month. Each group was housed separately under well hygienic with daily observation until the end of experiment.

SAMPLING:

Two blood samples were collected from each rabbit (5rabbits /group) at the 1st and 3rd day, 1st, 2nd and 3rd week post vaccination and/or drug administration for studying both cellular and humoral immune response. Sample 1: Whole blood (2-3ml) was collected from the ear vein in a sterile Wasserman tube containing heparin (0.5 mg/ml of blood) to be used for determination of phagocytic activity. Sample 2: In a sterile Wasserman tube, 3-5ml of blood was collected from the ear vein without an anticoagulant. The samples allowed to coagulate and then the serum was separated by centrifugation at 3000 rpm for 10 minutes and stored to -200ºC in sterile Eppendorf tubes until used for estimation of the serum total protein and for determination of serum nitric oxide and lysozyme activity.

ASSESSMENT OF CELLULAR IMMUNE RESPONSE:

I- Phagocytic activity[6]**:**

II- Measurement of serum nitric oxide level:

Nitric oxide level in the serum was measured according to the method described by[7].

III- Measurement of Lysozyme activity by agarose gel cell lysis assay:

The Lysozyme activity in the serum was measured according to the method described by[8].

Assessment of humoral immune response:

1) **Determination of Serum Total Protein, Albumin and Globulin:**

Serum total protein and albumin were determined by colorimetric method using commercial diagnostic kits. Globulins were determined by subtracting albumin from total protein level.

A- Estimation of serum total protein:

Estimation of serum total protein was carried out according to the Biuret method described by[9].

B- Estimation of serum albumin:

Estimation of serum albumin was carried out according to[10].

2) **Fractionation of serum proteins using Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique:** Qualitative fractionation of serum proteins for determination of serum Alpha, beta-and gamma-globulins was carried out using polyacrylamide gel columns according to the technique described by[11].

STATISTICAL ANALYSIS:

Immunological measurement were analyzed using repeated measures ANOVAs with treatment (control, vaccinated, vaccinated and treated, treated non vaccinated) as between subjects factors, and time point (of blood sample) as the within subjects factor. Both of them were done through the general linear models (GLM) procedure of the statistical package for Social Sciences version 21.0 (SPSS for Windows 2l.O.Inc.Chicago, IL, USA).

RESULTS:

I. EFFECT OF ATORVASTATIN ON CELLULAR IMMUNITY: 1- EFFECT ON PHAGOCYTIC ACTIVITY PERCENT (%): Concerning to the data present in table (1) represented to phagocytic percent, there are a significant increase in phagocytic percent in the vaccinated control group only after 7 days from starting the treatment, meanwhile after 14 and 21 days, phagocytic percent was highly significantly increased in treated-vaccinated, vaccinated control and treated non-vaccinated rabbits of groups (3, 2, 4), respectively compared with the non-vaccinated control group. It was clearly evident that the administration of atorvastatin to vaccinated group evoked a significant increase in phagocytic activity (%) at 7th , 14th and 21th days post drug administration (33.00±0.70, 65.80±0.66, 77.60±0.74, respectively) in comparison with vaccinated control group (33.40±0.50, 56.80±0.58, 68.20±0.37, respectively). A significant increase was also recorded in vaccinated control at 7th , 14th and 21th days post drug administration (33.40±0.50, 56.80±0.58, 68.20±0.37, respectively) compared with treated, non-vaccinated group (33.00±0.83, 47.00±0.44, 54.00±1.04, respectively). In addition vaccinated, treated group showed a significant increase in phagocytic activity (%) along 21 days of the experiment (33.00±0.70, 65.80±0.66, 77.60±0.74, respectively) when compared with treated, non-vaccinated group (33.00±0.83, 47.00±0.44, 54.00±1.04, respectively).

Table 1: Phagocytic percent (%) of rabbits in different groups 7, 14 and 21 days post treatment (n = 5, mean ± SE).

Parameters	Phagocytic %		
Durations / Groups	7 days	14 days	21 days
Non-vaccinated control	32.00^{b} ±0.54	37.00^{d} ±0.44	35.40^{d} ±0.92
Vaccinated control	33.40^{a} ±0.50	56.80^{b} ±0.58	68.20^{b} ±0.37
Treated vaccinated	33.00^{b}±0. 70	65.80^{a} ±0.66	77.60^{a} ±0.74
Treated non-vaccinated	33.00^{b}±0. 83	47.00^{c} ±0.44	54.00^{c} ±1.04
F test	*	**	**
LSD	0.88	5.20	7.68

- All data having different letters are differ significantly at p < 0.05.
- L S D: Least significant difference.
- *: Significant at 0.05 probability.
-**: Highly significant at 0.01 probability.

II.EFFECT ON PHAGOCYTIC INDEX:

The obtained data regarding the effect of atorvastatin administration on phagocytic index was summarized in table (2). In comparing with the non-vaccinated control group. There are a significant increase in phagocytic index in the vaccinated

control group only after 7 days from starting the treatment, meanwhile after 14 and 21 days, phagocytic index was highly significantly increased in treated-vaccinated, vaccinated control and treated non-vaccinated rabbits of groups (3,2,4), respectively. It was clearly evident that the administration of atorvastatin to vaccinated group evoked a significant increase in phagocytic index at 7th , 14th and 21th days post drug administration (3.70±0.07, 7.72±0.07, 9.12±0.05, respectively) in comparison with vaccinated control group (3.76±0.06, 6.22±0.08, 7.50±0.03, respectively). A significant increase was also recorded in vaccinated control at 7th , 14th and 21th days post drug administration (3.76±0.06, 6.22±0.08, 7.50±0.03, respectively) compared with treated, non-vaccinated group (3.34±0.15, 5.24±0.15, 6.68±0.15, respectively). Vaccinated, treated group showed a significant increase in phagocytic index along 21 days of the experiment (3.70±0.07, 7.72±0.07, and 9.12±0.05, respectively) when compared with treated, non-vaccinated group (3.34±0.15, 5.24±0.15, 6.68±0.15, respectively).

Table 2: Phagocytic index of rabbits in different groups 7, 14 and 21 days post treatment (n = 5, mean ± SE).

Parameters	Phagocytic index		
Durations / Groups	7 days	14 days	21 days
Non-vaccinated control	3.36^{b} ±0.08	4.04^{d} ±0.08	3.92^{d} ±0.05
Vaccinated control	3.76^{a} ±0.06	6.22^{b} ±0.08	7.50^{b} ±0.03
Treated vaccinated	3.70^{a} ±0.07	7.72^{a} ±0.07	9.12^{a} ±0.05
Treated non-vaccinated	3.34^{b} ±0.15	5.24^{c} ±0.15	6.68^{c} ±0.05
F test	*	**	**
LSD	0.14	0.66	0.91

- All data having different letters are differ significantly at p < 0.05.
- L S D: Least significant difference.
- *: Significant at 0.05 probability.
- **: Highly significant at 0.01 probability.

III.EFFECT ON SERUM NITRIC OXIDE LEVEL:

Concerning the effect of atorvastatin on serum nitric oxide level in vaccinated and non vaccinated rabbits, the obtained results shown in table (3) and clearly revealed that nitric oxide level was no significantly changes between all groups at first day of the experiment post atorvastatin treatment. On the other hand treated-vaccinated group showed the most highly significant increase in nitric oxide level compared with other groups for one week post vaccination especially at the 3rd days and 7th days (124.09±1.87,135.34±1.71, respectively) in comparison with vaccinated control group (102.92±1.12, 115.25±1.46, respectively) and treated, non-vaccinated group (94.76±2.26, 105.58±1.86, respectively). Moreover, nitric oxide level was significant increase between vaccinated control group at 3rd and 7th days of the experiment (102.92±1.12, 115.25±1.46, respectively) when compared with treated, non-vaccinated group (94.76±2.26, 105.58±1.86, respectively).

Table 3: Serum nitric oxide (µmol/L) of rats in different groups 1, 3 and 7 days post treatment (n = 5, mean ± SE).

Parameter	Nitric Oxide (µmol/L)		
Durations / Groups	1 day	3 days	7 days
Non-vaccinated control	83.54 ±1.94	80.93^{d} ±2.67	85.90^{d} ±4.17
Vaccinated control	81.24 ±5.23	102.92^{b} ±1.12	115.25^{b} ±1.46
Treated vaccinated	86.54 ±3.72	124.09^{a} ±1.87	135.34^{a} ±1.71
Treated non-vaccinated	82.54 ±6.54	94.76^{c} ±2.26	105.58^{c} ±1.86
F test	NS	**	**
LSD	4.59	7.76	8.89

- All data having different letters are differ significantly at p < 0.05.
- L S D: Least significant difference.
- N.S: Non significant changes.
- **: Highly significant at 0.01 probability.

IV.EFFECT ON SERUM LYSOZYME ACTIVITY:

The obtained data, presented in table (4) and illustrated that the administration of atorvastatin to vaccinated rabbits elicited no significant changes in serum lysozyme level at first days of the experiment between all rabbits groups. On the other hand treated-vaccinated group showed the most highly significant increase in serum lysozyme level compared with other groups for one week post vaccination especially at the 3rd days and 7th days (772.80±8.25, 835.40±33.31, respectively) in comparison with vaccinated control group (677.00±8.82, 723.00±34.93, respectively) and treated, non-vaccinated group (557.40±15.04, 616.80±32.62, respectively). Moreover, serum lysozyme level was significant increase between vaccinated control group at 3rd and 7th days of the experiment (677.00±8.82, 723.00±34.93, respectively) when compared with treated, non-vaccinated group (557.40±15.04, 616.80±32.62, respectively).

Table 4: Lysozyme activity (µg/ml) of rabbits in different groups 1, 3 and 7 days post treatment (n = 5, mean ± SE).

Parameter	Lysozyme Activity (µg/Ml)		
Durations / Groups	1 day	3 days	7 days
Non-vaccinated control	459.30 ±3.35	463.40^{d} ±2.13	459.80^{d} ±3.35
Vaccinated control	444.20 ±21.17	677.00^{b} ±8.82	723.00^{b} ±34.93
Treated vaccinated	443.20 ±16.57	772.80^{a} ±8.25	835.40^{a} ±33.31
Treated non-vaccinated	464.20 ±20.46	557.40^{c} ±15.04	616.80^{c} ±32.62
F test	NS	**	**
LSD	16.90	57.08	72.14

- All data having different letters are differ significantly at p < 0.05.
- L S D: Least significant difference.
- N.S: Non significant changes.
- **: Highly significant at 0.01 probability.

II. EFFECT OF ATORVASTATIN ON HUMORAL IMMUNITY:

A.EFFECT ON TOTAL SERUM PROTEIN, ALBUMIN AND GLOBULIN LEVELS: A-EFFECT ON TOTAL SERUM PROTEIN LEVELS:

It was obvious from table (5) that the administration of atorvastatin evoked no significant changes in total serum protein level at the first week between vaccinated and non-vaccinated groups. Vaccinated, treated group revealed a highly significant increase in total protein level all over the period of the experiment at 2nd and 3rd weeks (11.61±0.46, 13.25±0.69, respectively) when compared with the vaccinated control group (10.29±0.36, 11.54±0.53, respectively) and treated non-vaccinated group (9.23±0.20, 9.61±0.07, respectively). Total serum protein level was significant increase between vaccinated control group at 2nd and 3rd weeks of the experiment (10.29±0.36, 11.54±0.53, respectively) when compared with treated non-vaccinated group (9.23±0.20, 9.61±0.07, respectively).

Table 5: Serum total protein (g/dl) of rabbits in different groups 7, 14 and 21 days post treatment (n = 5, mean ± SE).

Parameter	Total Protein (G/Dl)		
Durations / Groups.	7 days	14 days	21 days
Non-vaccinated control	7.65 ±0.25	7.75^{d} ±0.15	7.50^{d} ±0.19
Vaccinated control	7.64 ±0.13	10.29^{b} ±0.36	11.54^{b} ±0.53
Treated vaccinated	7.75 ±0.27	11.61^{a} ±0.46	13.25^{a} ±0.69
Treated non-vaccinated	7.84 ±0.13	9.23^{c} ±0.20	9.61^{c} ±0.07
F test	NS	**	**
LSD	0.21	0.75	1.11

- All data having different letters are differ significantly at p < 0.05.
- L S D: Least significant difference.
- N.S: Non significant changes.
- **: Highly significant at 0.01 probability.

B.EFFECT ON SERUM ALBUMIN LEVEL:

Table (6) illustrated the percentage of serum albumin in vaccinated and non-vaccinated rabbits in response to Atorvastatin administration. The obtained data revealed that atorvastatin evoked a non significant difference in Serum albumin level in vaccinated group compared with vaccinated, non treated group; also no significant changes were detected at 1 and 2 weeks. While at 21st day of the experiment there was a significant decrease of serum Albumin level at treated vaccinated group (4.41±0.15) when compared with vaccinated control group (5.01±0.12) And treated non-vaccinated group (4.83±0.06).

Table 6: Serum albumin (g/dl) of rabbits in different groups 7, 14and 21 days post treatment (n = 5, mean ± SE).

Parameter	Albumin(G/Dl)		
Durations / Groups.	7 days	14 days	21 days
Non-vaccinated control	4.62 ±0.25	4.81 ±0.20	5.00^{a} ±0.21
Vaccinated control	4.43 ±0.22	5.03 ±0.06	5.01^{a} ±0.12
Treated vaccinated	4.56 ±0.28	4.94 ±0.20	4.41^{b} ±0.15
Treated non-vaccinated	4.76 ±0.35	5.13 ±0.06	4.83^{ab} ±0.06
F test	NS	NS	*
LSD	0.28	0.16	0.19

- All data having different letters are differ significantly at p < 0.05.
- L S D: Least significant difference.
- N.S: Non significant changes.
- *: Significant at 0.05 probability .

C.EFFECT ON SERUM GLOBULIN LEVEL:

It was obvious from table(7) that the administration of atorvastatin evoked no significant changes on serum globulin level between vaccinated and non vaccinated groups at first week of the experiment. On the other hand, vaccinated, treated group revealed a highly significant increase in serum globulin level in the 2nd and 3rd weeks during the period of the experiment (6.47±0.50. 8.84±0.75, respectively) when compared with the vaccinated control group (5.06±0.51, 6.32±0.45, respectively) and treated, non-vaccinated group (4.22±0.26, 4.78±0.09, respectively). A significant increase in vaccinated control group in the 2nd and 3rd weeks of the experiment (5.06±0.51, 6.32±0.45, respectively) compared with treated non-vaccinated group (4.22±0.26, 4.78±0.09, respectively).

Table 7: Total globulin (g/dl) of rabbits in different groups 7, 14 and 21 days post treatment (n = 5, mean ± SE).

Parameter	Total Globulin (G/Dl)		
Durations / Groups.	7 days	14 days	21 days
Non-vaccinated control	3.02 ±0.23	2.93^{c} ±0.20	2.49^{d} ±0.22
Vaccinated control	3.20 ±0.18	5.06^{b} ±0.51	6.32^{b} ±0.45
Treated vaccinated	3.18 ±0.14	6.47^{a} ±0.50	8.84^{a} ±0.75
Treated non-vaccinated	3.08 ±0.26	4.22^{b} ±0.26	4.78^{c} ±0.09
F test	NS	**	**
LSD	0.21	0.73	1.20

- All data having different letters are differ significantly at p < 0.05
- L S D: Least significant difference.
- N.S: Non significant changes.
- **: Highly significant at 0.01 probability.

2. EFFECT ON SERUM PROTEIN FRACTIONS (%) USING ELECTROPHORESIS: A-EFFECT ON ALPHA (A) GLOBULIN:

Table (8) illustrated the percentage of α globulin in vaccinated and non-vaccinated rabbits in response to atorvastatin administration. The obtained data revealed that atorvastatin evoked a non significant difference in α globulin percentage in

vaccinated group compared with vaccinated, non-treated group, also no significant changes were detected at 1, 2, and 3 weeks. On a similar ground, non-vaccinated, treated group showed no significant changes during 3 weeks of the experiment in comparison with non-vaccinated, non-treated group.

Table 8: Alpha globulin (g/dl) of rabbits in different groups 7, 14 and 21 days post treatment (n = 5, mean ± SE).

Parameter	A-Globulin (G/Dl)		
Durations / Groups.	7 days	14 days	21 days
Non-vaccinated control	0.85 ±0.025	0.93 ±0.022	0.92 ±0.061
Vaccinated control	0.83 ±0.056	0.92 ±0.058	0.89 ±0.050
Treated vaccinated	0.92 ±0.031	0.91 ±0.056	0.92 ±0.035
Treated non-vaccinated	0.82 ±0.046	0.91 ±0.052	0.90 ±0.047
F test	NS	NS	NS
LSD	0.05	0.04	0.044

- All data having different letters are differ significantly at p < 0.05.
- L S D: Least significant difference.
- N.S: Non significant changes.

B.EFFECT ON BETA (B) GLOBULIN:

The obtained results, summarized in the table (9), clearly demonstrated that non-significant changes in beta (β) globulin percentage of vaccinated group was induced by atorvastatin administration when compared with vaccinated, non-treated group. Also there were no significant changes in beta (β) globulin percentage of non-vaccinated, treated group when compared with non-vaccinated, non-treated control group.

Table 9: Beta globulin (g/dl) of rabbits in different groups 7, 14 and 21 days post treatment (n = 5, mean ± SE).

Parameter	B-Globulin (G/Dl)		
Durations / Groups.	7 days	14 days	21 days
Non-vaccinated control	0.56 ±0.051	0.61 ±0.029	0.54 ±0.064
Vaccinated control	0.57 ±0.019	0.53 ±0.038	0.51 ±0.078
Treated vaccinated	0.55 ±0.026	0.55 ±0.045	0.53 ±0.052
Treated non-vaccinated	0.49 ±0.023	0.57 ±0.038	0.57 ±0.043
F test	NS	NS	NS
LSD	0.035	0.040	0.060

- All data having different letters are differ significantly at p < 0.05.
- L S D: Least significant difference.
- N.S: Non significant changes.

C-EFFECT ON GAMMA (Γ) GLOBULIN:

The obtained results, summarized in table (10), clearly demonstrated that a significant increase gamma (γ) globulin percentage between vaccinated, treated group along 3 weeks of the study and vaccinated, non-treated group. On the other hand, present highly significant increase gamma (γ) globulin percentage between vaccinated, treated group along the course of the experiment (2.50±0.41, 5.01±0.46, 7.38±0.74, respectively) in comparison with the vaccinated control group (1.84±0.17, 3.61±0.46, 4.92±0.53, respectively). Moreover, there is significant increase gamma (γ) globulin percentage between vaccinated, treated group along the course of the experiment (2.50±0.41, 5.01±0.46, 7.38±0.74, respectively) in comparison with the treated, non-vaccinated group (1.64±0.11, 2.73±0.21, 3.31±0.11, respectively). Also vaccinated control group showed significant increase in gamma (γ) globulin percentage (1.84±0.17, 3.61±0.46, 4.92±0.53, respectively) when compared with treated, non-vaccinated group (1.64±0.11, 2.73±0.21, 3.31±0.11, respectively).

Table 10: Gamma globulin (g/dl) of rabbits in different groups 7, 14 and 21 days post treatment (n = 5, mean ± SE).

Parameter	Gamma-Globulin (G/Dl)		
Durations / Groups	7 days	14 days	21 days
Non-vaccinated control	1.57^{b} ±0.20	1.99^{c} ±0.26	1.02^{d} ±0.26
Vaccinated control	1.84^{ab} ±0.17	3.61^{b} ±0.46	4.92^{b} ±0.53
Treated vaccinated	2.50^{a} ±0.41	5.01^{a} ±0.46	7.38^{a} ±0.74
Treated non-vaccinated	1.64^{b} ±0.11	2.73^{bc} ±0.21	3.31^{c} ±0.11
F test	*	**	**
LSD	0.30	0.65	1.21

- All data having different letters are differ significantly at p < 0.05.
- L S D: Least significant difference.
- *: Significant at 0.05 probability.
- **: Highly significant at 0.01 probability.

DISCUSSION:

The present work was an attempt to explore the possible immune modulating effect of atorvastatin in both non vaccinated and RHDV-vaccinated rabbits by describing the effect of atorvastatin on both cellular and humoral immune response. Concerning to the effect of atorvastatin administration on the phagocytic activity % and phagocytic index, Treated-vaccinated, vaccinated control and treated non-vaccinated rabbits of groups show highly significant increase in phagocytic activity % and phagocytic index during the period of experiment when compared with non-vaccinated control group. Present investigation revealed a significant increase in vaccinated atorvastatin treated group when compared with vaccinated control group and also when compared with treated non-vaccinated group during experiment. On the same ground, a significant increase in vaccinated control group recorded during the experiment comparing with treated non-vaccinated group. These results are in accordance with that obtained by [12] who found that atorvastatin administration lead to enhance the phagocytic activity % and phagocytic index in mice[13] reported that atorvastatin given to rabbit at the dose of 10mg/kg lead to significant increase in phagocytic activity % and phagocytic index. Nitric oxide (NO) is a product of macrophages activated by cytokines, microbial compounds or both, is derived from the amino acid L-arginine by the enzymatic activity of inducible nitric oxide synthase (iNOS) and functions as a tumoricidal and antimicrobial molecule in vitro and in vivo[14]. Similarly,[15] found

that atorvastatin increased serum nitric oxide level when used therapeutic dose of atorvastatin 80mg/kg with healthy man. Moreover,[16] stated that atorvastatin increase nitric oxide level in serum of mice through its effect on thrombocyte. It had been provided [17] biochemical and functional evidence that atorvastatin promotes NO production by decreasing caveolin-1 expression in endothelial cells, regardless of the level extra-cellular LDL-cholesterol. Also oral therapeutic dose of atorvastatin increase serum nitric oxide level by decreasing ascorbate sensitive oxidants in human patient[18]. It have been reported[19] that serum lysozyme level increased by increasing dose of atorvastatin gradually from 10mg/kg to 80mg/kg. Similarly,[20] stated that atorvastatin increase lysozyme activity as all these results supported immuno-modulatory effect of atorvastatin. Lysozyme is a natural enzyme with antiviral, antibacterial and immune modulating actions that acts as a non specific defense mechanism and reflects the activities of macrophages[21]. Furthermore, [22] mentioned that serum total protein and globulin level increased in hyperlipidemic patient when treated with atorvastatin at dose of 40-80mg/kg on daily base. Atorvastatin has a direct effect on humoral immune response by increasing total serum protein and globulin level without causing renal injury[23]. On the same ground,[24] mentioned that atorvastatin increase serum total protein level when given for diabetic rats.[25] showed that atorvastatin increase serum total protein and high sensitivity c-reactive protein with reduction of total cholesterol level in high risk patients with atrial fibrillation. Also atorvastatin increase cellular and humoral immune response by increasing serum total protein and serum globulin levels which indicate a pleiotropic effect of atorvastatin[26]. On the same ground, treatment with atorvastatin in addition to a regimen with ACE inhibitors or ARBs may reduce proteinuria and the rate of progression of kidney disease in patients with chronic kidney disease, proteinuria and hypercholesterolemia[27]. While, [28] reported that atorvastatin has no significant change in serum albumin level when used lower dose of atorvastatin 10mg/kg with patients with chronic glomerulonephritis. Also[22] mentioned the same result as using atorvastatin with dose 40-80mg/kg induced no significant change in serum albumin level. The obtained result regarding the effect of atorvastatin on the level of serum total protein, albumin and globulin clearly illustrated that there was non-significant change in alpha and beta globulin percentages in vaccinated, treated group compared with vaccinated non-treated group in 2nd and 3rd weeks of the study. On the other hand, present highly significant increase gamma (γ) globulin percentage between vaccinated, treated group along the course of the experiment in comparison with the vaccinated control group. Moreover, there is significant increase gamma (γ) globulin percentage between vaccinated, treated group along the course of the experiment in comparison with the treated, non-vaccinated group. Also vaccinated control group showed significant increase in gamma (γ) globulin percentage when compared with treated, non-vaccinated group. In keeping with this line,[(22)] mentioned that serum total protein and globulin level increased in hyperlipidemic patient when treated with atorvastatin at dose of 40-80mg/kg on daily base. Similarly,[(29)] observed a significant increase gamma globulin after administration atorvastatin. These results were supported with that of[30] increase gamma globulin level with also increase production of antibody (IgG). Likewise, [31] observed that atorvastatin decrease high-sensitivity c-reactive protein with increase in gamma globulin in patient with multiple sclerosis.

CONCLUSION:

It could be concluded that from this study that atorvastatin has immunostimulant effect in immunity so it is advisable to use in rabbits either vaccinated or non-vaccinated.

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