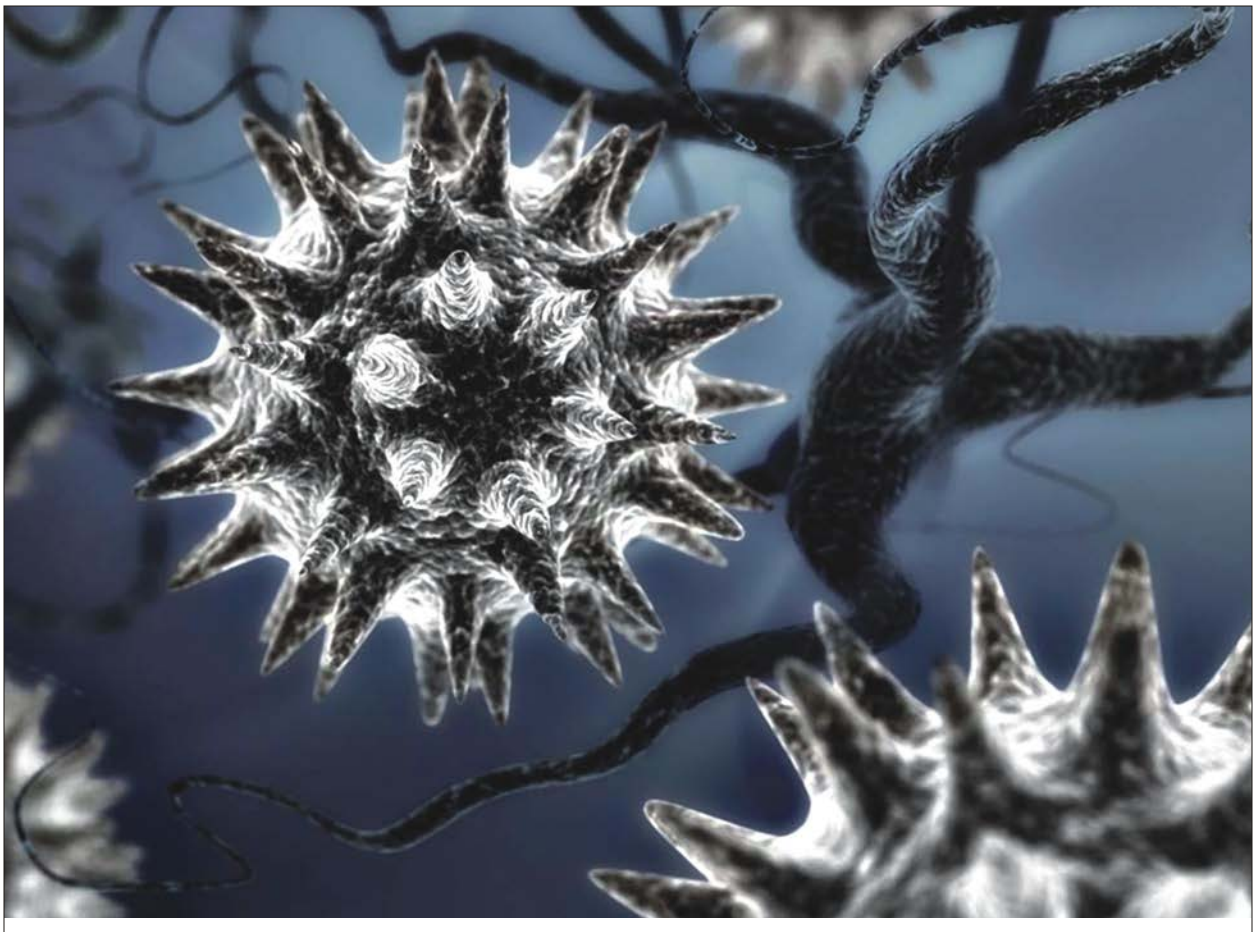

International Journal of Virology

ISSN 1816-4900

Academic Journals Inc.

www.academicjournals.com

International Journal of Virology 7 (4): 167-175, 2011
ISSN 1816-4900 / DOI: 10.3923/ijv.2011.167.175

Enhancing Effect of Ginseng Stem-leaf Saponins on the Immune Responses in Vaccinated Calves with FMD Bivalent Vaccine

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ABSTRACT

A comprehensive sero-immunological studies were conduced to reveal the adjuvant's effect of Ginseng Stem-leaf Saponins (GSLS) on the immune response of gel adjuvanted Bivalent Foot and Mouth Disease (FMD) vaccinated calves. These study conducted in two calve groups; group (A) vaccinated subcutaneously with bivalent Alhydragel adjuvanted (30)% FMD vaccine, while group (B) vaccinated subcutaneously with bivalent FMD vaccine adjuvanted with both Alhydragel and GSLS (10 mg/dose). The humeral and cellular immunoresponses were monitored in different tested groups that received the gel adjuvanted vaccine and the Alhydragel-GSLS adjuvanted vaccine. Results indicated that the higher immune responses were found in calves vaccinated with Alhydragel-GSLS adjuvanted vaccine up to 24 week while with Alhydragel alone was only up to 18 week.

Key words: Adjuvant, calves, FMD vaccine, GSLS, sero-immunological

INTRODUCTION

Foot and Mouth Disease (FMD) is an acute contagious viral disease of cloven footed animals (Radostits *et al*., 1995; Orsel *et al*., 2007). The causative agent is a single stranded positive-sense RNA virus that belongs to the genus Aphthovirus in the family Picornaviridae. There are seven immunologically distinct serotype of FMD virus, namely, O, A, C, Asia1, Sat1, Sat2 and Sat3 (Belsham, 1993). In Egypt, the disease is enzootic and outbreaks have been reported since 1950 (Mousa *et al*., 1974). Type O was the most prevalent since 1960 and onwards (Zahran, 1960; Daoud *et al*., 1988; Farag *et al*., 2005). Since 1950, 1953 and 1956 serotype A didn't recorded in Egypt (Zahran, 1960 recently serotype A FMD virus introduced to Egypt through live animals importation and the sever clinical signs occurred among cattle and buffaloes (Abed El-Rahman *et al*., 2006). The control of FMD in animals was considered to be important to effectively contain the disease in endemic areas, so that vaccination of animals is effective in limiting the spread of FMD (Nair and Sen, 1992) Most foot-and-mouth disease vaccines are made of BEI (binary Ethylenimine) inactivated virus that is adjuvanted with either aluminum hydroxide-saponin (AS) or oil adjuvant. Adjuvants, also can prolong the immune response and stimulate specific components of the immune response either humoral or cell mediated immunity (Dalsgarrd *et al*., 1990; Barnett *et al*., 2003; Pluimers, 2004; Lombard *et al*., 2007). Saponins extracted from Ginseng Stems and Leaves (GSLS) has an adjuvant effects on the immune responses of buffalo to vaccination against Foot-and-Mouth Disease Virus (FMDV) (Xie *et al*., 2004). This study was carried out as an attempt to detect the adjuvant effects of Saponin extracted

from ginseng stems and leaves (GSLS) on the immune responses of calves to vaccination against foot-and-mouth disease virus (FMDV) to improve local inactivated FMD vaccine.

MATERIALS AND METHODS

Animals: Nine calves (local breed) were clinically healthy and free from antibodies against FMD virus as proved by using SNT and ELISA were used in this study.

FMD viruses: FMD viruses O_1/3/93-Egypt Strain and A_1/Egypt/2006 are locally isolated strains of cattle origin. The viruses were typed at Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo and confirmed by Pirbright, International Reference Laboratories, United Kingdom.

FMD vaccines: Inactivated bivalent FMD vaccines were prepared using the local strains O_1/3/93 Egypt and A_1/Egypt/2006, propagated in BHK-21 cell line. The viruses had a titer of 10^8 $TCID_{50}$/mL for both and inactivated by Binary Ethylenemine (BEI), FMD vaccines with different adjuvant are formulized as follow:

Alhydragel: The inactivated FMD viruses suspension was mixed with 30% Alhydragel solution as adjuvant (Mousa *et al*., 1976).

Ginseng Stems and leaves (GSLS) Saponin: The inactivated FMD viruses suspension was mixed with 30% Alhydragel solution with adding 10 mg/dose of Ginseng stems and leaves saponin (Song and Hu, 2009).

Experimental design: Two groups each group contain 3 calves, were vaccinated with the tested vaccines beside unvaccinated group (3 calves). Serum samples were collected weekly post vaccination for one month then every 2 weeks post-vaccination till the end of experiment. The immune response was evaluated through the estimation of cellular and humoral immune level using Lymphocyte blastogenesis assay, SNT and ELISA.

Serum neutralization tet (SNT): It was performed using the technique as described by Ferreira (1976).

Enzyme linked immunosrobent assay (ELISA): It was carried out according to the method described by Voller *et al*. (1976).

Indirect solid phase ELISA was applied, patently prepared at department of FMD, Veterinary Serum and Vaccine Research Institute, Abbasia, Egypt.

Evaluation of cell-mediated immunity in vitro using lymphocyte Proliferation (3-(4,5-Dimethylthiazol-2-yl)-2,5-(MTT) Assay: It was applied according to Lucy (1984) following by modification adopted by El-Watany *et al*. (1999) and Abeer (2001).

RESULTS

Humoral immune response of calves vaccinated with FMD vaccines: Results of humoral immune response revealed that serum antibody protective titer evaluated by mean of SNT and ELISA were as follow:

1st group: Started at 2nd week post vaccination with the titers of 1.3 log_{10} by SNT and 1.5 by ELISA for O_1 was with titer of 1.4 log_{10} by SNT and 1.5 by ELISA for A_1. The highest level of antibody titers were at the 6th week post vaccination as 2.1 log_{10} by SNT and 2.4 by ELISA for O_1, as 2.2 log_{10} by SNT and 2.4 by ELISA for A_1 and the immunity duration lasted for 18 weeks post vaccination as 1.2 log10 by SNT and 1.5 by ELISA for O_1, as 1.2 log_{10} by SNT and 1.5 by ELISA for A_1

2nd group: Started at 2nd week post vaccination with the titers of 1.5 log_{10} by SNT and 1.6 by ELIS for O_1 and was with titer of 1.6 log_{10} by SNT and 1.6 by ELISA for A_1 The highest level of antibody titers were at the 8th week post vaccination as 2.4 log_{10} by SNT and 2.6 by ELISA for O_1, as 2.4 log_{10} by SNT and 2.6 by ELISA for A_1 and the immunity duration lasted for 24 weeks post vaccination as 1.2 log_{10} by SNT and 1.5 by ELISA for O_1, as 1.2 log_{10} by SNT and 1.5 by ELISA for A_1 Table 1-4

Evaluation of cell-mediated immunity in vitro using lymphocyte Proliferation (MTT) Assay: Obtained results of cell mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density) were as follow:

1st group: Delta Optical Density was (0.152-0.11-0.128) by using phytohaemagglutinin (PHA), Pokeweed (pok) mitogens and FMD virus at 3 day post vaccination and still rise reached its highest level (0.28-0.30-0.36) at 21 day post vaccination, then declined to (6 weeks)

Table 1: Immune status (SNT titer) of calves vaccinated with Alhydragel and modified Ginseng FMD vaccines against O_1/3/93-Egypt virus

	Type of vaccines								
	Alhydragel FMD vaccine				Ginseng FMD vaccine				
Weeks post vaccination	A_1	A_2	A_3	Average*	A_1	A_2	A_3	Average*	Control group of calves*
0	0.3	0.0	0.6	0.3	0.3	0.0	0.0	0.2	0.0
1	0.9	1.0	0.9	0.9	1.2	0.9	1.2	1.1	0.0
2	1.5	1.2	1.2	1.3	1.8	1.2	1.5	1.5	0.3
3	1.8	1.5	1.5	1.6	2.1	1.5	1.8	1.8	0.3
4	1.8	2.1	1.5	1.8	2.4	1.8	2.1	2.1	0.3
6	2.1	2.4	1.8	2.1	2.4	2.1	2.4	2.3	0.6
8	1.8	2.1	2.1	2.0	2.4	2.1	2.7	2.4	0.6
10	1.5	1.8	1.8	1.7	2.1	2.4	2.4	2.3	0.6
12	1.5	1.5	1.8	1.6	2.1	2.1	2.4	2.2	0.3
14	1.5	1.5	1.5	1.5	2.1	2.1	2.4	2.1	0.3
16	1.2	1.2	1.2	1.2	2.1	2.1	2.1	2.1	0.3
18	1.2	1.2	1.2	1.2	1.8	1.8	2.1	1.8	0.3
20	0.9	1.2	1.2	1.1	1.8	1.5	2.1	1.8	0.3
22	0.9	0.9	1.2	1.0	1.2	1.2	1.5	1.2	0.3
24	0.9	0.9	0.9	1.0	1.2	1.2	1.5	1.2	0.3
26	09	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.3
28	0.9	0.9	0.9	0.9	0.6	0.6	0.6	0.6	0.0
30	0.6	0.9	0.9	0.9	0.6	0.6	0.6	0.6	0.0

A_1, A_2 and A_3: vaccinated calves no. SNT; serum neutralization test. *: The results of SNT expressed as $log_{10}TCID_{50}$/mL. N.B.: The permissible protective level is 1.2 SNT titer

Table 2: Immune status (ELISA titer) of calves vaccinated with Alhydragel and modified ginseng FMD vaccines against O_1/3/93-Egypt virus

	Type of vaccines								
	Alhydragel FMD vaccine				Ginseng FMD vaccine				
Weeks post vaccination	A_1	A_2	A_3	Average*	A_1	A_2	A_3	Average*	Control group of calves*
0	0.6	0.3	0.9	0.9	0.6	0.3	0.3	0.6	0.3
1	1.0	1.2	1.0	1.0	1.5	1.2	1.5	1.4	0.3
2	1.5	1.5	1.5	1.5	2.1	1.5	1.8	1.8	0.3
3	2.1	1.8	1.8	1.9	2.1	1.8	2.1	2.0	0.3
4	2.1	2.1	1.8	2.0	2.7	2.1	2.4	2.4	0.6
6	2.4	2.7	1.8	2.3	2.7	2.4	2.7	2.6	0.9
8	2.1	2.4	2.1	2.2	2.7	2.4	3.0	2.7	0.9
10	1.8	2.1	1.8	1.9	2.4	2.4	2.4	2.4	0.9
12	1.8	1.8	1.8	1.8	2.4	2.1	2.4	2.3	0.6
14	1.8	1.8	1.8	1.8	2.1	2.1	2.4	2.2	0.6
16	1.5	1.8	1.5	1.6	2.1	2.1	2.4	2.2	0.6
18	1.5	1.5	1.5	1.5	2.1	1.8	2.1	2.0	0.6
20	1.2	1.5	1.2	1.3	1.8	1.5	2.1	1.8	0.6
22	0.9	0.9	1.2	1.0	1.8	1.5	1.8	1.8	0.3
24	0.9	0.9	1.2	1.0	1.2	1.2	1.5	1.3	0.3
26	09	0.9	0.9	0.9	1.2	0.9	1.2	1.1	0.3
28	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.3
30	0.6	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.3

A_1, A_2 and A_3: vaccinated calves no. *: The results of ELISA expressed as $\log_{10}$TCID $_{50}$/mL. N.B.: The permissible protective level is 1.5 ELISA titer

Table 3: Immune status (SNT titer) of calves vaccinated with Alhydragel and modified Ginseng FMD vaccines against A_1/Egypt/2006 virus

	Type of vaccines								
	Alhydragel FMD vaccine				Ginseng FMD vaccine				
Weeks post vaccination	A_1	A_2	A_3	Average*	A_1	A_2	A_3	Average*	Control group of calves*
0	0.3	0.0	0.6	0.3	0.3	0.0	0.0	0.2	0.0
1	0.9	1.2	0.9	1.0	1.2	1.2	1.2	1.2	0.0
2	1.5	1.5	1.2	1.4	1.8	1.5	1.5	1.6	0.3
3	1.8	1.5	1.5	1.6	2.1	1.5	1.8	1.8	0.3
4	1.8	2.1	1.5	1.8	2.4	1.8	2.1	2.1	0.3
6	2.1	2.4	2.1	2.2	2.4	2.1	2.4	2.3	0.6
8	1.8	2.1	2.1	2.0	2.4	2.1	2.7	2.4	0.6
10	1.8	1.8	1.8	1.8	2.1	2.4	2.4	2.3	0.6
12	1.5	1.8	1.8	1.7	2.1	2.4	2.4	2.3	0.3
14	1.5	1.5	1.5	1.5	2.1	2.1	2.4	2.1	0.3
16	1.2	1.2	1.2	1.2	2.1	2.1	2.1	2.1	0.3
18	1.2	1.2	1.2	1.2	1.8	2.1	2.1	2.0	0.3
20	0.9	1.2	1.2	1.1	1.8	1.5	2.1	1.8	0.3
22	0.9	0.9	1.2	1.0	1.2	1.5	1.5	1.4	0.3
24	0.9	0.9	0.9	1.0	1.2	1.2	1.5	1.2	0.3
26	09	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.3
28	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0
30	0.6	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0

A_1, A_2 and A_3: vaccinated calves No. SNT: serum neutralization test. *: The results of SNT expressed as $\log_{10}$ $TCID_{50}$/mL. N.B.: The permissible protective level is 1.2 SNT titer

Table 4: Immune status (ELISA titer) of calves vaccinated with Alhydragel and modified Ginseng FMD vaccines against A_1/Egypt/2006 virus

	Type of vaccines								
	Alhydragel FMD vaccine				Ginseng FMD vaccine				
Weeks post vaccination	A_1	A_2	A_3	Average*	A_1	A_2	A_3	Average*	Control group of calves*
0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0
1	1.2	1.5	1.2	1.3	1.8	1.5	1.8	1.7	0.0
2	1.8	1.5	1.5	1.6	2.1	1.8	1.8	1.9	0.3
3	2.1	2.1	1.8	2.0	2.4	2.1	2.4	2.3	0.3
4	2.1	2.4	2.1	2.3	2.7	2.4	2.7	2.6	0.3
6	2.4	2.7	2.4	2.6	3.0	2.7	3.0	2.9	0.6
8	2.4	2.4	2.4	2.4	3.0	2.7	3.0	2.9	0.6
10	2.1	2.1	2.1	2.1	2.7	2.7	3.0	2.8	0.6
12	2.1	2.1	2.1	2.1	2.4	2.1	2.4	2.3	0.3
14	1.8	1.8	1.8	1.8	2.1	2.1	2.4	2.2	0.3
16	1.8	1.8	1.8	1.8	2.1	2.1	2.4	2.2	0.3
18	1.5	1.5	1.5	1.5	2.1	2.1	2.4	2.2	0.3
20	1.2	1.5	1.2	1.3	2.1	1.8	2.4	2.1	0.3
22	1.2	1.5	1.2	1.3	1.8	1.8	2.1	1.9	0.3
24	0.9	1.2	1.2	1.1	1.8	1.5	1.8	1.7	0.3
26	09	0.9	0.9	0.9	1.5	1.2	1.2	1.2	0.3
28	0.9	0.9	0.9	0.9	1.2	0.9	0.9	1.0	0.0
30	0.6	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0

A_1, A_2 and A_3: vaccinated calves No. *: The results of ELISA expressed as $\log_{10}$ $TCID_{50}$/mL N.B.: The permissible protective level is 1.5 ELISA titer

2nd group: Delta Optical Density was (0.232-0.191-0.309) by using (PHA) (pok) and FMD virus at 3 day post vaccination and still rise reached its highest level (0.413-0.442-0.524) at 21st day post vaccination, then declined after (9 weeks) (Table 1-4).

DISCUSSION

The control of FMD in animals was considered to be important to effectively contain the disease in endemic areas, so that vaccination of animals is effective in limiting the spread of FMD.

So, this study is to improvement of inactivated FMD Alhydragel vaccine with adding Ginseng stems and leaves saponin as an adjuvant.

From Table 1 and 2 the results revealed that SNT and ELISA titers for Alhydragel FMD vaccines, go in hand with the results obtained are consistent with the statement of Hamblin *et al.* (1986) who explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion, while ELISA probably measure all classes of antibodies even those produced against incomplete and non-infectious virus.

From Table 3 and 4 the results revealed that SNT and ELISA titers for Alhydragel and Ginseng FMD vaccine agreed with Rivera *et al.* (2003), Hu *et al.* (2003), Sun *et al.* (2005), Yan *et al.* (2007) and Song and Hu (2009). who showed that adjuvant properties of Ginseng Extract as potent adjuvant induced higher antibody titers than the vaccine adjuvanted with $Al(OH)_3$ and improved the potency of adjuvants. Results supported also by Scaglione *et al.* (1996), Rivera *et al.* (2003) and Wang *et al.* (2009) who found that ginseng might help the vaccine work more effectively, increasing antibody production.

Table 5: Cell-mediated Immune response of calves Vaccinated with Alhydragel and modified Ginseng FMD vaccines

Time post vaccination		Calves vaccinated with Alhydragel FMD vaccine				Calves vaccinated with Alhydragel and Ginseng FMD vaccine				Control group of calves*
		1	2	3	Average*	1	2	3	Average*	
Prevaccination	PHA	0.070	0.070	0.073	0.071	0.074	0.076	0.078	0.076	0.060
	POK	0.012	0.011	0.016	0.013	0.023	0.024	0.025	0.024	0.011
	V	0.018	0.020	0.025	0.021	0.048	0.049	0.050	0.049	0.020
3rd day	PHA	0.148	0.150	0.158	0.152	0.230	0.232	0.234	0.232	0.070
	POK	0.107	0.108	0.115	0.110	0.190	0.191	0.192	0.191	0.015
	V	0.125	0.127	0.132	0.128	0.308	0.309	0.310	0.309	0.022
1st Week	PHA	0.178	0.180	0.182	0.180	0.293	0.294	0.295	0.294	0.070
	POK	0.123	0.124	0.128	0.125	0.201	0.203	0.205	0.203	0.012
	V	0.195	0.197	0.202	0.198	0.340	0.340	0.343	0.341	0.026
2nd Week	PHA	0.257	0.258	0.265	0.260	0.358	0.360	0.362	0.360	0.080
	POK	0.228	0.229	0.236	0.231	0.339	0.341	0.343	0.341	0.014
	V	0.312	0.313	0.317	0.314	0.488	0.490	0.492	0.490	0.027
3rd Week	PHA	0.278	0.279	0.283	0.280	0.412	0.412	0.415	0.413	0.090
	POK	0.302	0.303	0.307	0.304	0.441	0.440	0.445	0.442	0.019
	V	0.359	0.360	0.364	0.361	0.523	0.522	0.527	0.524	0.028
4th Week	PHA	0.252	0.253	0.257	0.254	0.294	0.296	0.298	0.296	0.010
	POK	0.314	0.315	0.319	0.316	0.357	0.360	0.363	0.360	0.011
	V	0.322	0.321	0.326	0.323	0.560	0.558	0.562	0.560	0.027
5th Week	PHA	0.221	0.222	0.227	0.224	0.292	0.294	0.296	0.294	0.090
	POK	0.264	0.263	0.268	0.265	0.333	0.332	0.337	0.334	0.015
	V	0.282	0.283	0.283	0.284	0.428	0.430	0.432	0.430	0.024
6th Week	PHA	0.225	0.224	0.229	0.226	0.282	0.283	0.287	0.284	0.080
	POK	0.248	0.250	0.254	0.251	0.266	0.264	0.268	0.266	0.016
	V	0.237	0.236	0.241	0.238	0.339	0.340	0.344	0.341	0.023
7th Week	PHA	0.198	0.199	0.203	0.200	0.254	0.252	0.256	0.254	0.070
	POK	0.218	0.219	0.223	0.220	0.241	0.240	0.245	0.242	0.012
	V	0.197	0.198	0.202	0.199	0.319	0.320	0.324	0.321	0.021
8th Week	PHA	0.120	0.118	0.122	0.120	0.197	0.197	0.200	0.198	0.080
	POK	0.131	0.131	0.134	0.132	0.229	0.230	0.234	0.231	0.013
	V	0.149	0.149	0.152	0.150	0.300	0.300	0.303	0.301	0.023
9th Week	PHA	0.099	0.100	0.101	0.100	0.206	0.204	0.208	0.206	0.070
	POK	0.173	0.175	0.177	0.175	0.251	0.253	0.255	0.253	0.011
	V	0.114	0.116	0.118	0.116	0.268	0.270	0.272	0.270	0.020
10th Week	PHA	0.098	0.100	0.102	0.100	0.132	0.134	0.136	0.134	0.070
	POK	0.133	0.135	0.137	0.135	0.180	0.180	0.183	0.181	0.011
	V	0.114	0.116	0.118	0.116	0.160	0.163	0.168	0.165	0.020

*: Δ Optical densities, **: Type of mitogen, PHA: Phytohaemagglutinin, V: FMD Virus, POK: Pokeweed, N.B.: The permissible protective level is 0.250 delta optical density

From Table 5, the results of evaluation of cell mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density). Supported by Knudsen *et al.* (1979) and Sharma (1981) who reported that cell mediated immune response was a constitute of immune response against FMD virus. And in agreement in some points with Garcia-Valcarcel *et al.* (1996) El-Watany *et al.* (1999) and Abeer (2001). Mansour (2001) and Samir (2002) that FMD vaccine stimulated the cellular immune response and lymphocyte stimulation by

FMDV was greater than by mitogens (PHA) and (POK) and appeared increased in 1st and 2nd weeks post vaccination. While disagreed with El-Watany *et al.* (1999) and Mansour (2001) in that cell mediated immune response reach its highest level on the 14th day.

The obtained results were in agreement with Song *et al.* (2002), Chen *et al.* (2008) and Sun *et al.* (2009) who stated that Ginseng extract act as an activator of the TH1 response. The Th_1 type is characterized by the production of antigen-specific IgG2a a Th_1 nd the secretion of gamma interferon, interleukins which favor cellular immunity.

Our results also were supported by (Xie *et al.*, 2004) and Wang *et al.* (2007) who mentioned that Ginseng extract enhanced interleukins which enhance cell mediated immune response.

Finally, it can conclude that: The usage of Saponin extracted from ginseng stems and leaves in inactivated FMD vaccine gave long lasting immunity than that which with Alhydragel adjuvant alone GSLS and improve both cellular and humoral immunity and gave earlier and more long lasting immunity.

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