Immunomodulating Effects of Isoprinosine in Vaccinated Rabbit

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Abstract

The object of this study was to investigate the effects of oral administration of Isoprinosine (163.3mg/ kg) for 21 days, in rabbit before vaccination with (RHVD) vaccine on cellular and humoral immune response. Forty rabbits were divided into four equal groups. 1st group was left without treatment and used as control, 2nd group was treated with oral dose of isoprinosine (163.3 mg/kg) for 21 days, 3rd group was vaccinated with rabbit haemorrhagic viral disease (RHVD) vaccine and the 4th group was orally administered with isoprinosine (163.3 mg/kg) for 21 days then was vaccinated with (RHVD) vaccine. Results proved that vaccinated treated rabbits showed a significant improvement in phagocytic percent, phagocytic index, lysozyme activity, nitric oxide, serum total protein, serum albumin, gamma globulins in comparison with vaccinated non-treated rabbits. DNA destruction percent observed in liver and kidney tissue samples of treated, vaccinated and treated vaccinated groups. In conclusion, it appears that isoprinosine improves the cellular immune response in vaccinated rabbits and it has a genotoxic effect on hepatocytes of vaccinated rabbits.

Keywords: Isoprinosine, Immunity, RHDV, genotoxicity

Introduction

Vaccination failure causes severe money losses to rabbit farm owner and farmers especially during handling, transportation and administration. So many researchers tried to overcome these problems by using antiviral agents either by feeding or administration. Inosine pranobex (Methisoprinol, ISO, Isoprinosine) is an immuno-modulatory antiviral drug that has been licensed since 1971 in several countries worldwide. It has been reported to exert antiviral and antitumour activities *in vivo* which are secondary to an immunomodulating effect, and early results suggest beneficial clinical effects in several diseases and infections including mucocutaneous herpes simplex infections, subacute sclerosing panencephalitis, genital warts, influenza, zoster, and type B viral hepatitis, as well as in homosexual men with persistent generalised lymphadenopathy ***(Campoli-Richards et al., 1986).***

Isoprinosine has been reported to be beneficial for HIV-infected patients not only due to the stimulation of the immune system but also due to its effect on folate synthesis being helpful against *Pneumocystis jiroveci (Pedersen et al., 1990).* Isoprinosine has been reported to be effective in subacute sclerosing panencephalitis (SSPE) which is a chronic encephalitis occurring after infection with measles virus. *(Gutierrez et al., 2010).* The oral dose in mucocutaneous herpes simplex is 1 g four times daily for 7 to 14 days. An oral dose of 1 g three times daily is given for 14 to 28 days as an adjunct to standard topical treatment for genital warts. In subacute sclerosing panencephalitis, the oral dose is 50 to 100 mg/kg daily in divided doses given every 4 h *(Tobólska et al., 2018).*

This study was designed to explore possible immune-modulating effects of isoprinosine on immune response in vaccinated and non-vaccinated rabbits. In addition, to throw light on genotoxicity effect on liver and kidney of rabbits.

Materials and methods
Drug and vaccine
Isoprinosine is the proprietary trademark of Newport Pharmaceuticals International, Inc. Isoprinosine 50 mg/ kg (Ip50, reference standard) *(Yapo et al., 2011).* This dose converted to rabbit dose by surface area ratios of some common laboratory species and man to (163.3 mg/kg P.O. once daily) *(Paget and Barnes, 1964).*

Inactivated rabbit haemorrhagic disease virus vaccine was used for active immunization of experimental rabbits. RHVD vaccine was purchased from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt, and was given subcutaneously in a dose of 0.5 ml for each rabbit *(Hanaa et al., 2009).*

Animals
A total of forty (40) Newzealand white rabbits of 2-3 months old and weighing about 1800-2000 gm were used in the work. They were obtained from Laboratory Animal Farm, Faculty of Veterinary Medicine, Zagazig University. They hadn't a history of RHVD outbreaks or vaccination against RHVD. The rabbits were tested serologically and confirmed to be zero-negative for RHVD.

Experimental design
Rabbits were distributed randomly into 4 groups as: 1st group (control group) left non-treatment and non-vaccination, 2nd group (vaccinated group) vaccinated by inactivated (RHDV) vaccine which given subcutaneously in a dose of 0.5 ml for each rabbit, 3rd group (isoprinosine) treated by oral administration of isoprinosine (163.3 mg/ kg) which given for 3 weeks and 4th group (treatment and vaccination) treated by isoprinosine

which given orally for 3 weeks then vaccinated by inactivated (RHDV) vaccine which given subcutaneously in a dose of 0.5 ml for each rabbit. The blood samples were collected to determine of phagocytic percent and phagocytic index. The serum samples were collected to determined lysozyme activity, nitric oxide production, total protein, albumin, alpha-, beta- and gamma-globulins. The tissue samples (kidney and liver) were taken for comet test.

Assessment of cellular immune response:

Determination of lysozyme activity in the serum measured according to the method described by *Schltz (1987)* at 1st, 2nd and 3rd day post vaccination. Determination of nitric oxide level in the serum was measured as the method showed by *Rajarman et al. (1998) and Ramadan and Attia (2003)* at 1st, 2nd and 3rd day post vaccination. Determination phagocytic activity in blood samples was measured according to the method described by *Wilkinson (1977) and Lucy and Larry (1982)* at 7th day post vaccination.

Assessment of humoral immune response:

Determination of total protein was carried out according to the Biuret method described by *Weichselbaum (1946) and Gornal et al. (1949)* at 7th, 14th and 21th day post vaccination. Estimation of serum albumin was carried out according to *Dumas et al. (1971)* at 7th, 14th and 21th day post vaccination.

Qualitative fractionation of serum proteins using sodium dodecyle sulfate polyacrylamide gel electrophoresis technique for determination of serum alpha-, beta- and gamma-globulins was carried out according to the technique described by *Daves (1964) and Ornstein (1964)* at 7th, 14th and 21th day post vaccination.

Assessment of genotoxicity by the single cell gel electrophoresis (SCGE)/ comet assay:

The comet assay was carried out according to the technique described by *Singh et al. (1988).*

Statistical analysis:

The obtained data in the present study were statistically analyzed using the computer program (SPSS version 15 for Windows), and comparison were made using one-way ANOVA. Post hock test was carried which considered statistically significant when $P < 0.05$ *(Snedecor and Cochran, 1982).*

Results
Effect of isoprinosine on cellular immune response
Phagocytic activity

It was cleared from table (1) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced high significant increase

in the mean values of phagocytic percent at 7th day post vaccination (74.00 ± 1.155) in comparison with vaccinated non treated group (60.00 ± 1.155). Vaccinated group produced high significant increase in the mean value of phagocytic percent at 7th day post vaccination (60.00 ± 1.155) in comparison with control group (non-treated non-vaccinated group) (53.00 ± 0.577). On the other hand, it was cleared from table (1) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced high significant increase in the mean values of phagocytic index at 7th day post vaccination (4.63 ± 0.088) in comparison with vaccinated non treated group (3.37 ± 0.088). Vaccinated group produced high significant increase in the mean value of phagocytic index at 7th day post vaccination (3.37 ± 0.088) in comparison with control group (non-treated non-vaccinated group) (2.60 ± 0.058).

Lysozyme activity

It was cleared from table (1) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced high significant increase in the mean values of serum lysozyme activity at 1st, 2nd and 3rd day post vaccination (17.28 ± 0.518, 16.42 ± 1.270 and 17.13 ± 0.53, respectively) in comparison with vaccinated group (7.72 ± 0.482, 10.15 ± 0.104 and 12.44 ± 0.756, respectively). Vaccinated group produced high significant increase in the mean values of serum lysozyme activity at 1st, 2nd and 3rd day post vaccination (7.72 ± 0.482, 10.15 ± 0.104 and 12.44 ± 0.756, respectively) in comparison with control group (non-treated non-vaccinated group) (3.63 ± 0.617, 3.04 ± 0.373 and 3.44 ± 0.325, respectively).

Nitric oxide production

It was cleared from table (1) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced high significant increase in the mean values of serum nitric oxide production at 1st, 2nd and 3rd days post vaccination (2.48 ± 0.104, 2.24 ± 0.059 and 2.54 ± 0.145, respectively) in comparison with vaccinated non treated group (1.33 ± 0.064, 1.83 ± 0.029 and 2.00 ± 0.038, respectively). Vaccinated group produced high significant increase in the mean values of serum nitric oxide production at 1st, 2nd and 3rd day post vaccination (1.33 ± 0.064, 1.83 ± 0.029 and 2.00 ± 0.038, respectively) in comparison with control group (non-treated non-vaccinated group) (0.74 ± 0.191, 0.74 ± 0.084 and 0.74 ± 0.076, respectively).

Effect of isoprinosine on humoral immunity

Serum total protein concentration

It was illustrated from table (1) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in

the mean values of serum total protein at 7th and 21th day post vaccination (7.47 ± 0.481, 7.83 ± 0.338, respectively) in comparison with vaccinated non treated group (6.50 ± 0.153, 7.53 ± 0.260, respectively).

Oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced high significant decrease in the mean values of serum total protein at 14th day post vaccination (8.10 ± 0.116) in comparison with vaccinated non treated group (7.30 ± 0.208).

Vaccinated group produced high significant increase in serum total protein at 7th, 14th and 21th post vaccination (6.50 ± 0.153, 7.30 ± 0.208 and 7.53 ± 0.260, respectively) in comparison with control group (non-treated non-vaccinated group) (4.40 ± 0.666, 5.10 ± 0.208 and 4.67 ± 0.145, respectively).

Serum albumin level

It was illustrated from table (1) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of serum albumin at 7th and 21th day post vaccination (3.20 ± 0.116, 4.20 ± 0.058, respectively) in comparison with vaccinated non treated group (4.27 ± 0.521, 4.45 ± 0.260, respectively).

Oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced a significant decrease in the mean values of serum albumin at 14th day post vaccination (3.47 ± 0.120) in comparison with vaccinated non treated group (4.50 ± 0.306).

Vaccinated group produced high significant increase in the mean values of serum albumin at 7th, 14th and 21th post vaccination (4.27 ± 0.521, 4.50 ± 0.306 and 4.45 ± 0.260, respectively) in comparison with control group (non-treated non-vaccinated group) (3.83 ± 0.504, 3.87 ± 0.176 and 3.83 ± 0.088, respectively).

Serum globulins level

It was illustrated from table (1) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of serum globulins level at 7th, 14th and 21th day post vaccination (3.30 ± 0.10, 4.13 ± 0.088 and 3.87 ± 0.377, respectively) in comparison with vaccinated non treated group (3.20 ± 0.116, 3.63 ± 0.203 and 3.80 ± 0.20, respectively).

Vaccinated group produced high significant increase in the mean values of serum globulins at 7th, 14th and 21th post vaccination (3.20 ± 0.116, 3.63 ± 0.203 and 3.80 ± 0.20, respectively) in comparison with control group (non-treated non-vaccinated group) (2.17 ± 0.167, 2.73 ± 0.033 and 2.83 ± 0.067, respectively).

Alpha-1 globulin level

It was illustrated from table (1) that oral administrated of isoprinosine

(163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of serum alpha-1 globulins level at 7th, 14th and 21th day post vaccination (1.51 ± 0.034, 1.79 ± 0.117 and 1.59 ± 0.075, respectively) in comparison with vaccinated non treated group (1.52 ± 0.052, 1.59 ± 0.013 and 1.65 ± 0.038, respectively).

Vaccinated group produced non-significant change in the mean values of serum alpha-1 globulins at 7th, 14th and 21th day post vaccination (1.52 ± 0.052, 1.59 ± 0.013 and 1.65 ± 0.038, respectively) in comparison with control group (non-treated non-vaccinated group) (1.45 ± 0.026, 1.58 ± 0.079 and 1.62 ± 0.044, respectively).

Alpha-2 globulin level

It was illustrated from table (1) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of serum alpha-2 globulins level at 7th, 14th and 21th day post vaccination (0.77 ± 0.038, 0.88 ± 0.034 and 0.94 ± 0.043, respectively) in comparison with vaccinated non treated group (0.71 ± 0.066, 0.89 ± 0.055 and 0.89 ± 0.02, respectively).

Vaccinated group produced non-significant change in the mean values of serum alpha-2 globulins at 7th, 14th and 21th day post vaccination (0.71 ± 0.066, 0.89 ± 0.055 and 0.89 ± 0.02, respectively)

in comparison with control group (non-treated non-vaccinated group) (0.74 ± 0.081, 0.92 ± 0.038 and 0.92 ± 0.062, respectively).

Beta globulin level

It was illustrated from table (1) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of beta globulin level at 7th, 14th and 21th day post vaccination (0.68 ± 0.024, 0.93 ± 0.433 and 0.85 ± 0.069, respectively) when compared with vaccinated non treated group (0.68 ± 0.116, 0.78 ± 0.039 and 0.80 ± 0.09, respectively).

Vaccinated group produced high significant increase in the mean values of beta globulin level at 7th, 14th and 21th day post vaccination (0.68 ± 0.116, 0.78 ± 0.039 and 0.80 ± 0.09, respectively) in comparison with control group (non-treated non-vaccinated group) (0.55 ± 0.064, 0.72 ± 0.021 and 0.83 ± 0.024, respectively).

Gamma globulin level

It was showed from table (1) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced a significant decrease in the mean values of gamma globulin level at 7th and 14th day post vaccination (0.40 ± 0.017 and 0.52 ± 0.015, respectively) when compared with vaccinated non-treated group (0.52 ± 0.018 and 0.62 ± 0.018, respectively).

Oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non- significant decrease in the mean values of gamma globulin level at 21th day post vaccination (0.68 ± 0.015) when compared with vaccinated non-treated group (0.81 ± 0.019).

Vaccinated group produced high significant increase in the mean values of gamma globulin level at 7th, 14th and 21th day post vaccination (0.52 ± 0.018, 0.62 ± 0.018 and 0.81 ± 0.019, respectively) in comparison with control group (non-treated non-vaccinated group) (0.32 ± 0.062, 0.45 ± 0.032 and 0.47 ± 0.091, respectively).

Comet test (apoptosis assay) for Kidney samples

It was illustrated from table (2) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced high significant decrease in the mean values of comet % at 21th day post vaccination (18.03 ± 0.145) when compared with vaccinated non treated group (19.23 ± 0.285).

Vaccinated group produced high significant increase in the mean values of comet % at 21th day post vaccination (19.23 ± 0.285) in comparison with control group (non-treated non-vaccinated group) (13.40 ± 0.306).

It was illustrated from table (2) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of tail length at 21th day post vaccination (4.87 ± 0.419) when compared with vaccinated non treated group (5.41 ± 0.520).

Vaccinated group produced non-significant increase in the mean values of tail length at 21th day post vaccination (5.41 ± 0.520) in comparison with control group (non-treated non-vaccinated group) (6.04 ± 0.313).

It was illustrated from table (2) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of % DNA in tail at 21th day post vaccination (11.81 ± 1.786) when compared with vaccinated non treated group (9.29 ± 1.163).

Vaccinated group produced non-significant change in the mean values of % DNA in tail at 21th day post vaccination (9.29 ± 1.163) in comparison with control group (non-treated non-vaccinated group) (11.71 ± 0.649).

It was illustrated from table (2) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of tail moment (severity of damage) at 21th day post vaccination (0.44 ± 0.065) when compared with vaccinated non treated group (0.92 ± 0.391).

Vaccinated group produced a significant change in the mean values of tail moment (severity of

damage) at 21th day post vaccination (0.92 ± 0.391) in comparison with control group (non-treated non-vaccinated group) (0.73 ± 0.075).

It was illustrated from table (2) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of olive moment at 21th day post vaccination (1.29 ± 0.223) when compared with vaccinated non treated group (1.71 ± 0.491).

Vaccinated group produced non-significant change in the mean values of olive moment at 21th day post vaccination (1.71 ± 0.491) in comparison with control group (non-treated non-vaccinated group) (1.38 ± 0.082).

Comet test (apoptosis assay) for liver samples

It was illustrated from table (2) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced high significant increase in the mean values of comet % at 21th day post vaccination (16.63 ± 0.203) when compared with vaccinated non treated group (14.33 ± 0.285).

Vaccinated group produced high significant increase in the mean values of comet % at 21th day post vaccination (14.33 ± 0.285) in comparison with control group (non-treated non-vaccinated group) (12.13 ± 0.353).

It was illustrated from table (2) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of tail length at 21th day post vaccination (6.83 ± 0.781) when compared with vaccinated non treated group (7.10 ± 0.301).

Vaccinated group produced a significant increase in the mean values of tail length at 21th day post vaccination (7.10 ± 0.301) in comparison with control group (non-treated non-vaccinated group) (4.45 ± 0.163).

It was illustrated from table (2) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of % DNA in tail at 21th day post vaccination (7.89 ± 0.630) when compared with vaccinated non treated group (6.14 ± 0.678).

Vaccinated group produced non-significant change in the mean values of % DNA in tail at 21th day post vaccination (6.14 ± 0.678) in comparison with control group (non-treated non-vaccinated group) (6.38 ± 0.753).

It was illustrated from table (2) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced a significant increase in the mean values of tail moment (severity of damage) at 21th day post vaccination (0.56 ± 0.065) when compared with vaccinated non treated group (0.40 ± 0.033).

Vaccinated group produced a significant increase in the mean values of tail moment (severity of damage) at 21th day post vaccination (0.40 ± 0.033) in comparison with control group (non-treated non-vaccinated group) (0.23 ± 0.024).

It was illustrated from table (2) that oral administrated of isoprinosine (163.3 mg/kg) to vaccinated rabbits produced non-significant change in the mean values of olive moment at 21th day post vaccination (1.03 ± 0.083) when compared with vaccinated non treated group (0.80 ± 0.068).

Vaccinated group produced non-significant change in the mean values of olive moment at 21th day post vaccination (0.80 ± 0.068) in comparison with control group (non-treated non-vaccinated group) (0.75 ± 0.087).

Table (1): *The effects of isoprinosine (163.3 mg/ kg P.O once daily for 21 successive days on immune responses in vaccinated rabbits (mean $\pm$ S.E), (N=10):*

Assessment of cellular immune response	Day of investigation	Control	Treated	Vaccinated	Treated vaccinated
Lysozyme activity (µg/ ml)	1st day	3.63 ± 0.617	7.00 ± 0.738^a	7.72 ± 0.482^b	17.28 ± 0.518^{cdf}
	2nd day	3.04 ± 0.373	8.54 ± 0.404^a	10.15 ± 0.104^b	16.42 ± 1.270^{cdf}
	3rd day	3.44 ± 0.325	10.10 ± 0.174^a	12.44 ± 0.756^{be}	17.13 ± 0.531^{cdf}
Nitric oxide level (µmol/ l)	1st day	0.74 ± 0.191	1.31 ± 0.103^a	1.33 ± 0.064^b	2.48 ± 0.104^{cdf}
	2nd day	0.74 ± 0.084	1.52 ± 0.068^a	1.83 ± 0.029^{be}	2.24 ± 0.059^{cdf}
	3rd day	0.74 ± 0.076	1.74 ± 0.038	2.00 ± 0.038^b	2.54 ± 0.0145^{cdf}
Phagocytic percent	7th day	53.00 ± 0.577	66.33 ± 0.882^a	60.00 ± 1.155^{be}	74.00 ± 1.155^{cdf}
Phagocytic index	7th day	2.60 ± 0.058	3.93 ± 0.088^a	3.37 ± 0.088^{be}	4.63 ± 0.088^{cdf}
Assessment of humoral immune response	Day of investigation	Control	Treated	Vaccinated	Treated vaccinated
Total protein (g/ dl)	7th day	4.40 ± 0.666	5.43 ± 0.203^a	7.47 ± 0.481^{be}	6.5 ± 0.153^c
	14th day	5.10 ± 0.208	6.33 ± 0.203^a	8.10 ± 0.116^{be}	7.30 ± 0.208^{cdf}
	21th day	4.67 ± 0.145	7.33 ± 0.260^a	7.83 ± 0.338^b	7.53 ± 0.260^c
Albumin (g/ dl)	7th day	3.83 ± 0.504	2.37 ± 0.176^a	4.27 ± 0.521^e	3.20 ± 0.116
	14th day	3.87 ± 0.176	2.57 ± 0.348^a	4.50 ± 0.306^e	3.47 ± 0.120^{cdf}
	21th day	3.83 ± 0.088	2.43 ± 0.376^a	4.45 ± 0.260^e	4.20 ± 0.058^d
Globulin (g/dl)	7th day	2.17 ± 0.167	3.07 ± 0.033^a	3.20 ± 0.116^b	3.30 ± 0.100^c
	14th day	2.73 ± 0.033	3.77 ± 0.549^a	3.63 ± 0.203^b	4.13 ± 0.088^c
	21th day	2.83 ± 0.067	3.90 ± 0.306^a	3.80 ± 0.200^b	3.87 ± 0.377^c
α₁-globulin (g/dl)	7th day	1.45 ± 0.026	1.47 ± 0.021	1.52 ± 0.052	1.51 ± 0.034
	14th day	1.58 ± 0.079	1.69 ± 0.061	1.59 ± 0.013	1.79 ± 0.117
	21th day	1.62 ± 0.044	1.69 ± 0.117	1.65 ± 0.038	1.59 ± 0.075
α₂-globulin (g/dl)	7th day	0.74 ± 0.081	0.81 ± 0.050	0.71 ± 0.066	0.77 ± 0.038
	14th day	0.92 ± 0.038	0.90 ± 0.056	0.89 ± 0.055	0.88 ± 0.034
	21th day	0.92 ± 0.062	1.00 ± 0.155	0.89 ± 0.020	0.94 ± 0.043
β-globulin (g/dl)	7th day	0.55 ± 0.064	0.66 ± 0.064^a	0.68 ± 0.116^b	0.68 ± 0.024^c
	14th day	0.72 ± 0.021	0.80 ± 0.058^a	0.78 ± 0.039^b	0.93 ± 0.433^c
	21th day	0.73 ± 0.024	0.76 ± 0.041^a	0.80 ± 0.09^b	0.85 ± 0.069^c
γ-globulin (g/dl)	7th day	0.32 ± 0.062	0.10 ± 0.015^a	0.52 ± 0.018^{be}	0.40 ± 0.017^{df}
	14th day	0.45 ± 0.032	0.25 ± 0.035^a	0.62 ± 0.018^{be}	0.52 ± 0.015^{df}
	21th day	0.47 ± 0.091	0.36 ± 0.035	0.81 ± 0.019^{be}	0.68 ± 0.015^{cd}

Means carrying the different letter within the same raw were significantly different; (P< 0.05). S.E: Standard error of mean. ([a]) Significant difference between control and treated, ([b]) significant difference between control and vaccinated, ([c]) significant difference between control and treated vaccinated, ([d]) significant difference between treated and treated vaccinated, ([e]) significant difference between treated and vaccinated and ([f]) significant difference between vaccinated and treated vaccinated.

Table (2): *The effects of Isoprinosine (163.3 mg/ kg P.O once daily for 21 successive days on (Genotoxicity using the comet assy) (apoptosis assay) for kidney and liver samples of vaccinated rabbits (mean ± S.E):*

Kidney samples	Day of investigation	Control	Treatment	Vaccinated	Treated vaccinated
Comet %	21th day	13.40 ± 0.306	17.10 ± 0.153^a	19.23 ± 0.285^{be}	18.03 ± 0.145^{cdf}
Tail length (px)	21th day	6.04 ± 0.313	10.62 ± 0.073^a	5.41 ± 0.520^e	4.87 ± 0.419^d
% DNA in tail	21th day	11.71 ± 0.649	8.83 ± 0.397	9.29 ± 1.163	11.81 ± 1.786
Tail moment	21th day	0.73 ± 0.075	1.11 ± 0.041	0.92 ± 0.391	0.44 ± 0.065^d
Olive moment	21th day	1.38 ± 0.082	1.63 ± 0.186	1.71 ± 0.491	1.29 ± 0.223
Liver samples	**Day of investigation**	**Control**	**Treatment**	**Vaccinated**	**Treated vaccinated**
Comet %	21th day	12.13 ± 0.353	16.17 ± 0.219^a	14.33 ± 0.285^{be}	16.63 ± 0.203^{cf}
Tail length (px)	21th day	4.45 ± 0.163	6.85 ± 1.124^a	7.10 ± 0.301^b	6.83 ± 0.781^c
% DNA in tail	21th day	6.38 ± 0.753	8.17 ± 0.850	6.14 ± 0.678	7.89 ± 0.630
Tail moment	21th day	0.23 ± 0.024	0.48 ± 0.029^a	0.40 ± 0.033^b	0.56 ± 0.065^{cf}
Olive moment	21th day	0.75 ± 0.087	0.86 ± 0.068	0.80 ± 0.068	1.03 ± 0.083^c

Means carrying the different letter within the same raw were significantly different; (P< 0.05). S.E: Standard error of mean. ([a]) Significant difference between control and treated, ([b]) significant difference between control and vaccinated, ([c]) significant difference between control and treated vaccinated, ([d]) significant difference between treated and treated vaccinated, ([e]) significant difference between treated and vaccinated and ([f]) significant difference between vaccinated and treated vaccinated.

Discussion

To ensure the efficacy of RHVD vaccine, we should administrate immunostimulating drug to overcome vaccination failure. The rabbit haemorrhagic disease (RHD) virus, belonging to the Caliciviridae family, causes high mortality in farm and wild rabbits *(Meyers et al., 1991, Abd El-khalek and Hady, 2007 and Niedzwiedzka, 2008).* Isoprinosine was found to have a broad spectrum of antiviral activity, inhibiting the RNA viruses, influenza (INFV) and parainfluenza (PIV), as well as the DNA viruses, herpes simplex (HSV) and vaccinia (VACV). Isoprinosine in vivo caused a statistically significant increase in survival of treated animals (hamster, mice) infected with RNA or DNA viruses. This effect of Isoprinosine was apparent in animals which were previously immunosuppressed *(Ohnishi et al., 1983).*

The study is designed to investigate the immunomodulating effects of oral administration of isoprinosine on vaccinated rabbits with RHVD vaccine. The parameters considered for this concept included cellular immune response as (phagocytic percent, phagocytic index, serum lysozyme activity and serum nitric oxide production and humoral immune response as serum total protein, serum albumin, serum total globulins, serum alpha-, beta and gamma globulins and genotoxic effect on percent of DNA destruction).

The results of the present work reported that oral administration of isoprinosine (163.3 mg/ kg) for 21 successive days illustrated high significant increase in the mean values of phagocytic activity percent and phagocytic index on 7th day post vaccination in comparison with vaccinated non treated group and normal rabbits (non-vaccinated and non-treated). Phagocytic cell is a cell that engulf and digests debris and invading microorganisms, macrophage consider a large phagocyte. Phagocyte acts as storage for lysozyme, myeloperoxidase acid hydrolysis and complement activators *(Farthman and Schoffel, 1998).* The increase in phagocytic activity percent and phagocytic index express the positive immune effects of isoprinosine (163.3 mg/ kg). These results were in agreement with *Wybran et al. (1978)* who observed that isoprinosine significantly enhanced phagocytosis at concentrations of 50, 100, 300, and 500µg/ml. This indicated that isoprinosine may improve the phagocytic capacity of granulocytes which may be beneficial to the host defense against invading microorganisms in vitro leucocyte assays which were studied in normal individuals. *Wybran et al. (1982)* proved that inosine pranobex is an immuno-modulating agent which potentiates T-lymphocyte and phagocytic cell function. *Ghram et al. (1989)* suggested that isoprinosine is helpful in reversing

the suppressive effect of BRD viruses on peripheral blood mononuclear cells. They revealed that isoprinosine enhanced immunity by influence on lymphocytes, monocytes, and macrophages. They found that it increased immunological responses in normal as well as immune-depressed animals in viral infections. *Mulacahy et al. (1991)* observed that methisoprinol increase expression of membrane Fc and C receptors involved in macrophage phagocytosis of microorganisms. *FlØ et al. (1994)* found that isoprinosine stimulated granulocyte oxidative metabolism which measured by chemiluminescence. Isoprinosine in range from 250 to 1000 µg/ml increased the chemiluminescence of granulocytes and maximal response was observed at 4000 µg/ml. Moreover, Isoprinosine in concentration from 100 to 500 µg/ml increases the proportion of monocytes able to phagocytose yeast cells and also augments lymphokine induced macrophage proliferation. *Kostro et al. (2002)* found that isoprinosine stimulates the phagocytic activity of macrophages and neutrophils cellular and cell-free immune responses. They found that isoprinosine increases proliferation of T lymphocytes, activates Th and Tc cells, stimulates IL-1 and IL-2 production, and increases the activity of INF-α and INF-γ. *Siwicki et al. (2003)* found that

methisoprinol at a concentration of 100 microl/ ml modulated and restored the phagocyte and lymphocyte activity of head kidney suppressed by VHSV. *Wojcik et al. (2004)* found that isoprinosine is a better stimulator by determination percentage of phagocytes and phagocytic index in carbaryl intoxicated turkeys which vaccinated with Newcastle Disease (ND) virus. *Siwicki et al. (2008)* observed immune-modulating influence of methisoprinol on the pronephros macrophages and lymphocytes after suppression induced by infectious haematopoietic necrosis virus (IHNV). They observed the possibility of methisoprinol to eliminate the depressive influence of viral infection on the cell-mediated immunity responses. *Siwicki et al. (2014)* found that respiratory burst activity and potential killing activity of the pronephros phagocytes were significantly higher in eels fed the diet with methisoprinol than in those fed the control.

Lysozyme activity is consider as a part of non-specific immune response and exists in leukocytes. Human lysozyme is present in lysozymes of phagocytic cells, granulocytes and monocytes *(Burgess et al., 1994).* Lysozyme is a natural enzyme acting as antimicrobial and had immune-modulating actions. It acts as a non-specific defense mechanism and reflects the activities of

macrophages *(El-Sayed and Manal., 2007).* So the activation of phagocytic activity will be reflected on serum lysozyme activity.

In the present work, it has been observed that oral administrated of Isoprinosine (163.3 mg/ kg) for 21 successive days elicited high significant increase in the mean values of serum lysozyme activity on 1st, 2nd and 3th days post vaccination in comparison with vaccinated non treated rabbits and normal rabbits (non-vaccinated and non-treated). These results were in agreement with *Saurabh and Sahoo (2008)* who examined that lysozyme activity increases after supplementation with a wide range of immunostimulants in various fish species. *Siwicki et al. (2014)* proved that methisoprinol increased the level of lysozyme activity by examining the influence of dietary supplementation with the synthetic compound methisoprinol 200 mg/ kg for four weeks on selected nonspecific immune parameters in intensively cultured juvenile European eel (Anguilla anguilla).

In the present study, oral administrated of Isoprinosine (163.3 mg/kg) for 21 successive days displayed high significant increase in the mean values of serum nitric oxide on 1st, 2nd and 3th days post vaccination in comparison with vaccinated non treated rabbits and normal rabbits (non-vaccinated non-treated). These results were in agreement with *Wessely-Szponder and Kloc (2010)* who they reported that isoprinosine in higher doses caused excessive release of enzymes, generation of free radicals and NO by neutrophils which may cause lung injury and worsening symptoms of bovine respiratory disease (BRD) in calves.

Nitric oxide (NO) and TNF are two keys mediators that play a role in host defense and inflammatory response. They regulate each other *(Yamamoto and Gaynor, 2001; Wu et al., 2003).* Which was in the same direction with that obtained by *Lasek et al. (2015)* who found that isoprinosine enhanced TNF-a secretion significantly (in short-term-24-hour and prolonged term-72-hour cultures) in patients with a depressed function of the immune system. *Petrova et al. (2010)* who observed an increase in serum level of tumor necrosis factor-alpha (TNF-alpha) at 7th to 10th day in 10 healthy adult volunteers who received Isoprinosine 1g, 3 times daily, 5 consecutive days weekly. All of that, proved the ability of Isoprinosine to activate the production of Nitric oxide. Nitric oxide (NO) is a product of macrophages which is activated by cytokine microbial compounds which is derived from amino acid l-arginine by the enzymatic activity of inducible nitric oxide synthetase (iNOS) *(Nathan, 1992).* Nitric oxide gets involved in broad spectrum of processes. These included the differentiation, proliferation and apoptosis of immune cells, the production of

cytokines and other soluble mediators, the expression of co-stimulatory and adhesion molecules, and the synthesis and deposition of extracellular matrix components *(Marshall et al., 2000)*. These evidences proved that, Isoprinosine has ability to produce a significant increase in cytokines production which in the same direction with that obtained by *Milano et al. (1991); Neumann et al. (2001); Kostro et al. (2002); Li et al. (2006); Magnadóttir (2010); Petrova et al. (2010) and Lasek et al. (2015).*

The obtained results regarding the effects of oral administration of isoprinosine on the level of serum total protein and albumin illustrated a significant decrease at 14th day post vaccination when compared with vaccinated non-treated rabbits. These results disagree with that of *Wojcik et al. (2004)* who evaluated the ability of isoprinosine in carbaryl intoxicated turkeys which vaccinated with a live attenuated Roakin strain of the Newcastle disease to stimulate total protein immunity of the suppressed turkeys to a significant extent than with birds having proper immunity systems. *Tykałowski et al. (2009)* who demonstrated that methisoprinol administered to turkey embryos in ovo on day 26 of incubation at doses of 5, 10 or 20 mg per embryo did not induce any disturbances in the hatching process. In addition, it was shown not to exert any negative effect on total protein and albumin contents of blood serum of the 5-day-old turkey poults. *Siwicki et al. (2014)* who found that total protein was significantly higher in eels fed the methisoprinol diet. *Bhandary et al. (2017)* who administered mice with isoprinosine (2000 mg/kg body weight) daily for 28 consecutive days. In the subacute toxicity studies, they found that isoprinosine hasn't abnormalities in terms of hematological and biochemical parameters in sub-acute toxicity tests (total protein and albumin). These disagreements might be due to difference in doses, duration of treatment and model of experiment. Reduction in plasma proteins and albumin therefore has a tendency to reveal persistent damage of hepatocytes *(Rahman et al., 2000)* which proved later by the results of comet test on hepatocytes in our study.

The obtained data displayed a non-significant change in the level in globulins, alpha-1 globulins, alpha-2 globulins, beta-globulins at 7th, 14th and 21th days post vaccination in vaccinated treated rabbits when compared with vaccinated non-treated rabbits. Oral administration of Isoprinosine produced high significant increase in the level in globulins, alpha-1 globulins, alpha-2 globulins, beta-globulins at 7th, 14th and 21th days when compared with non-vaccinated non-treated rabbits which in the same line of *Carstens et al. (1995)* who evaluated the use of the biologic

immune activation markers neopterin and β2-microglobulin in monitoring human immunodeficiency virus (HlV)-positive patients without acquired immunodeficiency syndrome (AIDS) treated with Isoprinosine and placebo. They found that, the concentrations of β2-microglobulin and neopterin had increased both in the Isoprinosine group and the placebo group.

The obtained data examined a significant decrease in gamma globulin value in vaccinated treated group at 7th and 14th day post vaccination when compared with vaccinated non-treated rabbits. These results were in same line of *Hyllseth et al. (1992)* who found that isoprinosine and combination groups had lower CIEP and γ-globulin values at some early and late testing points of an experiment lasting 12 weeks which was carried out to examine whether these two drugs (two dosage levels each) separately or in combination could influence the pathogenesis of infection with Aleutian disease virus (ADQ) in sapphire (Aleutian-au) mink. The reduction which observed in gamma globulin values may be due to effect of isoprinosine on lymphoid organs which consider the main part in synthesis of gamma globulin *(Cooper et al., 1969).*

Nitric oxide (NO) has primarily cytotoxic and cytostatic activity *(Meurs et al., 2003)* so the increase in nitric oxide production in vaccinated treated rabbits reflects the increase in DNA destruction percent in treated and vaccinated treated rabbits and reflect the cytotoxicity and cytostatic ability of Isoprinosine. In the present investigation, it had been observed that isoprinosine induced a remarkable increase in DNA destruction (%) and tail moment (severity of damage) in vaccinated treated rabbits compared with vaccinated non-treated rabbits indicated a genotoxic effect of isoprinosine (163.3 mg/kg) on the DNA of hepatocytes after 21 successful days post vaccination.

Isoprinosine didn't induce a remarkable increase in DNA destruction (%) and tail moment (severity of damage) in vaccinated treated rabbits compared with vaccinated non-treated rabbits indicated a non-genotoxic effect of isoprinosine (163.3 mg/kg) on the DNA of podocytes after 21 successful days post vaccination. These results disagree with that of *Bhandary et al. (2017)* who evaluated the safety of isoprinosine by acute ad subacute toxicity studies. They administered mice with (2000 mg/ kg body weight) isoprinosine, they found that no mortality or signs of acute toxicity was observed in the mice. The results did not show any treatment related abnormalities in terms of hematological and biochemical parameters in sub-acute toxicity tests. No significant differences in body weight and organ weight between the control and treated

groups were observed. Histopathological analysis showed no morphological changes of the vital organs namely liver and kidney between the control and treated groups. ***Tobólska et al. (2018)*** who used BALB/3T3 clone A1 and HepG2 cells which were incubated with inosine pranobex at concentrations from 0.1 to 1000 μg/mL. They found that inosine pranobex did not induce a significant dose-related increase in DNA damage in either cell line (A1 and HepG2) at concentrations from 0.1 to500 μg/ml. But, there was a concentration-dependent increase with statistical significance in the number of comets in cells incubated with inosine pranobex at concentrations of 1000 μg/ml. They concluded that inosine pranobex is not mutagenic in the Salmonella typhimurium reverse mutation assay. The difference in results may be due to long duration of treatment (21 days) and different genotoxociy effects of isoprinosine and its metabolites on different organs of animal, which agree with ***Wybran et al., (1978) and Wybran et al. (1982)*** who demonstrated that, the major excretion product of inosine is uric acid other components of it undergo oxidation and glucuronidation, and the metabolites are excreted in the urine.

Conclusion

It could be concluded that using a dose of (163.3 mg/kg) isoprinosine for 21 successive days before vaccination by RHVD augmented the immune status by potentiation the cellular immune response in addition to its obvious genotoxic effect on hepatocytes of rabbits so it is be recommended to be used in rabbits. This concluded give a reason to think about how overcome the genotoxicity of Isoprinosine to sheer this combination economic wild.

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