



Research Article

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Experimental Study of *M. oleifera* Leaf Extract in Aqueous and Powder Formulations on Immune Response of Chicks by Nitroblue Tetrazolium Reduction Test

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Abstract

In the present study, the aqueous extract and dried powder of *Moringa oleifera* leaf was investigated for its immunostimulatory effect in broiler chick. It was evaluated on the basis of percentage of formazan positive cell in blood samples and skin sample collected at the end of reaction. Fifty (50 no.) day-old chicks were kept into 5 groups each contain 10 chicks. First two treatment groups (T₁ and T₂) were fed with aqueous extract and dried powder of *M. oleifera* respectively each @ 250 mg/kg b.wt. For comparison, a levamisole fed group T₃ was included, to compare the immunomodulatory effect of the preparations of *M. oleifera* with that of a known positive immunomodulator, which is fed @ 10 mg/kg b.wt. The other two groups T₄ and T₅ included in the experimental design served as the vaccinated and unvaccinated control groups respectively. The effect of treatment was recorded from day 14th day. From day 14 up to day 42, *M. oleifera* treated groups showed stimulatory effect on non-specific immune response which is judged by relative percentage of formazan positive cells in blood sample. No mortality was recorded in the treated groups. *M. oleifera* showed stimulatory effect on non-specific immune response in chicks.

Keywords: Formazan positive cells, Immunostimulatory effect, *Moringa oleifera*

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1. Introduction

The botanical name of Moringa is *Moringa oleifera*. It belongs to family *Moringaceae*. The Moringa plant originated initially in the northern part of India and soon move to southern part. The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal purpose. A number of Indian

medicinal plants and various rasayans have been claimed to have immunomodulatory activities. Moringa is one of the important plant mentioned in all medicinal herbs (Ganguly and Prasad, 2010; Prasad and Ganguly, 2012). The aqueous extract of the mature flowers of *M. oleifera* contain free natural sugars, D-mannose and D-glucose in the ratio of 1:5 and two unidentified carbohydrate bearing material along with protein and ascorbic acid (Pramanik and Islam, 1998). The leaves of Moringa also contain same compound as well as niazirin and niazirin (Faizi et al., 1994). The leaves have high protein content of 27% and are rich in vitamins A and C, calcium, iron and phosphorus. The Bureau of plant industry, in its report, stated that weight per weight, Moringa leaves have the calcium equivalent to 4 glasses of milk, the vitamin C content of 7 oranges, potassium of 3 bananas, 3 times the iron of spinach, 4 times the amount of vitamin A in carrots, and 2 times the protein in milk (Prasad and Ganguly, 2012). The present study was carried out to assess and evaluate the effect of *M. oleifera* leaf in non-specific immune response in broiler chicks.

2. Materials and Methods

The plant was procured locally and herbal preparations are made. Aqueous extract was made on boiling known amount of Moringa leaf in water and dried powder was made on shadow drying. Fifty (50 No.) day-old Vencobb chicks were procured and maintained under standard farm conditions in Department of Veterinary Microbiology, Faculty of Veterinary Science & Animal Husbandry, BAU, Ranchi. The chicks were divided into 5 equal groups each containing 10 chicks. The treatment given to the chicks under the 5 groups was as follows:

Group 1: The chicks were treated with aqueous extract of *Moringa oleifera* leaves @ 250mg/kg b.wt. and were vaccinated accordingly as per the vaccination schedule.

Group 2: The chicks were treated with leaf powder of *M. oleifera* @ 250mg/kg b.wt. and were vaccinated accordingly as per the vaccination schedule.

Group 3: The chicks were treated with levamisole@10mg/kg b.wt. orally and were vaccinated accordingly as per the vaccination schedule.

Group 4: The chicks of this group were not fed with any extract of *M. oleifera* and were vaccinated accordingly as per the vaccination schedule. This group served as positive control.

Group 5: The chicks were neither fed any extract of *M. oleifera* nor were vaccinated against Newcastle disease (ND). This group served as control.

Non-specific immune response of neutrophil and monocyte of circulating blood was estimated by nitroblue tetrazolium reduction test. In a similar way non-specific immune response of macrophages was also be assessed by tissue section collected during delayed hypersensitivity reaction with the same method as per Culling et al. (1985) with certain modifications. Neutrophils were separated from blood and suspension of neutrophils was made in Hanks Balanced Salt solution (HBSS). Aliquot of suspension was mixed with equal volume of 0.2% NBT in PBS. The aliquots were mixed and incubated for 1 h at 37°C. A wet smear was prepared and cells were counted for the presence of black formazan granules. The percentage of cell containing formazan granules as well as size of granules were used as marker of non-specific immune response (Culling et al., 1985). All data were subjected to statistical analysis as per standard methods outlined by Snedecor and Cochran (1994).

3. Results and Discussion

In the present study, effect of *Moringa oleifera* on non-specific immune response was estimated by counting formazan positive cells in blood samples collected at weekly interval. Significantly higher formazan positive cells were observed in levamisole treated group from day 21 to day 42 which is due to increased response to antigen and mitogen and activity of effectors like lymphocytes (Table). Both *in vivo* and *in vitro* studies have confirmed levamisole as a good potentiator of both specific and non-specific immune response (Adams, 1995; Prasad et al., 2011).

Table. Formazan count in blood sample in the treatment groups

Age (day)	T ₁	T ₂	T ₃	T ₄	T ₅
1	21±0.577 ^a	21±0.577 ^a	21±0.577 ^a	21±0.577 ^a	21±0.577 ^b
7	20±0.966 ^a	20±0.966 ^a	20±0.966 ^a	20±0.966 ^a	20±0.966 ^{ab}
14	25±0.930 ^b	26±0.577 ^b	28±0.930 ^b	24±0.577 ^b	20±0.730 ^{ab}
21	26±0.577 ^b	26±0.966 ^b	29±0.365 ^b	24±0.577 ^b	20±0.730 ^{ab}
28	26±0.577 ^b	26±0.577 ^b	29±0.258 ^b	24±0.365 ^b	20±0.730 ^{ab}
35	27±0.577 ^b	28±0.577 ^b	32±0.730 ^c	25±0.577 ^b	19±1.390 ^{ab}
42	32±0.775 ^c	31±0.577 ^c	35±0.966 ^d	26±0.931 ^b	18±0.577 ^a

The values bearing different superscripts in a row differed significantly.

Each value was expressed as Mean ± SE, (P<0.05)

The present study showed the higher value of formazan positive cells in moringa treated groups T₁ and T₂ as compared to control group (Table) which might be due to stimulatory effect of *Moringa oleifera* on non-specific immune response. The findings in the present study was in agreement with Gupta *et al.* (2010) who worked on immunomodulatory effect of ethanolic extract of *M. oleifera* leaves in normal and immunosuppressed mice model and reported that the *M. oleifera* treated group shows a significant rise (P<0.05) in phagocytic index which indicates the rise in non-specific immune response.

4. Conclusion

From the results obtained from the present study, it was recommended that *M. oleifera* in optimum formulated combination or dosage can safely recommended as a potential immunostimulant.

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