ABSTRACT

**Aim:** The aim of the study was to determine the immunopotentiatory effect of Stresomix, a polyherbal anti stress, immunomodulatory formulation against blue tongue vaccination. The hypothesis was that antistress, adaptogenic and immunomodulatory properties of Stresomix constituent herbs viz. *Magnifera indica*, *Withania somnifera* and *Phyllanthus emblica* would enhance the immune response in deccani lambs and sheeps.

**Study Design:** Total 12 lambs and 12 adult deccani sheep were selected for the study. Vaccination was done with inactivated pentavalent Blue tongue vaccine at 1ml per animal subcutaneously.

**Place and Duration of Study:** The experiment was conducted in Central Research Institute for Dry Land Agriculture, Santoshnagar, Hyderabad, India in Deccani breed of sheeps.

**Methodology:** The parameters viz. antibody titre and serum biochemical parameters were studied statistically.
1. INTRODUCTION

Small ruminants such as goats and sheep are important sources of financial security for farmers [1]. Sheep have significant role in economy. According to the 1972 census, the country had 40 m sheep, which contributed approximately $175 m (Rs 1400 m) per year to the national economy, based on a rough estimate of production of 34.3 m kg of wool, 101 m kg of mutton, and 14.6 m skins [2]. According to the live stock census of 2003 the country second in respect of goat and third in respect of sheep population in the world [3]. In India, sheeps are primarily maintained on natural pasture lands / waste lands with in situ grazing and often exposed to various stress like nutrition, physiological and environmental stress [4]. Stress evokes harmful responses that interferes with the general health, productivity and result in alteration in the process of homeostasis, metabolism and immunosuppression [5]. Variation in feed intake, protein deficiency, starvation, shortage of feeding space, vaccination and various other managemental operations may lead to stress which ultimately cause immunosuppression [6,7]. Stress cause of reduction in performance and increase susceptibility to disease [8]. Therefore it is mandatory to minimize the stress and restore the immunity in livestock for optimum production by adopting proper managemental practice and or supplementation of immunomodulators. Hence, an attempt was made to study the efficacy of herbal immunomodulators and antistressor-Stresomix (M/S Ayurvet Limited, India) in potentiating immune response in Deccani lambs and sheep vaccinated with inactivated multivalent blue tongue virus.

2. MATERIALS AND METHODS

The experiment was conducted in Central Research Institute for Dry Land Agriculture, Santoshnagar, Hyderabad, India in Deccani breed of sheeps. A total of 12 lambs (142±8.1 days old) with mean body weight of 14.8±0.23 kg and 12 adult sheeps (408±13.7 days old) with mean body weight of 28.3±0.41 kg were selected for the experiments. The animals were drenched against all parasites and after thorough examination for good health allowed to acclimatize to the conditions for a period of 7 days prior to commencement of experimentation. Animals were supplemented with 150 and 250g of concentrate mixture (CP: 18 and TDN: 70%) per day per animal, respectively. In addition, mineral bricks were provided in the pens.

2.1 Experiment I

Lambs were divided into group I: control group (n=6) without any treatment and group II (n=6) lambs were supplemented with Stresomix @ 1 kg/tonne of concentrate mixture for 5 days pre and 5 days post vaccination.

2.2 Experiment II

Similarly, the adult sheeps were divided into two groups group I: control group (n=6) without any treatment and group II (n=6) sheeps were supplemented with Stresomix @ 1 kg/tonne of concentrate mixture for 5 days pre and 5 days post vaccination.

Vaccination was done with inactivated pentavalent Blue tongue vaccine @1ml per animal subcutaneously. At day 28 post vaccination serum samples were collected from individual animals and antibody titters in serum samples were assessed with competitive enzyme linked immuno sorbent assay according to Jochim protocol [9]. The antibody titters in serum samples less than 50 are considered as seronegative and more than 50 as seropositive. Serum samples were further analyzed for metabolites like albumin, blood urea nitrogen (BUN) and creatinine and also transaminase (SGOT and SGPT) using Qualigens Diagnostic kits.

**Results:** In deccani lambs, increase in the antibody titre against blue tongue (BT) in Stresomix supplemented group II (89.08) was significantly (P<0.01) higher as compared to lambs in untreated group I (69.88). Similarly, In adult deccani sheep, significant (P<0.01) increase in antibody titre was obtained in Stresomix supplemented group II (84.57) in comparison of untreated control group I (57.88)

**Conclusion:** The antibody titre determination and serum biochemical analysis revealed that the Stresomix did not change the biochemical profile so it is safe to use Stresomix for improving post vaccination immune response in sheep

Keywords: Immuno potentiator; poly herbal; antibody titre; serum biochemical profile.
2.3 Statistical Analysis

All the results were analyzed statistically by using student's 't' test as per Snedecor and Cochran [10].

3. RESULTS AND DISCUSSION

3.1 Antibody Titre in Deccani Lambs

At day 0 there was no significant difference in the antibody titre of the lambs in both the untreated and treated group. Significant increase in the antibody titre was observed at day 28 post vaccination in both the groups, but the antibody titre against blue tongue (BT) of Stresomix supplemented group II (89.08) was significantly (P<0.01) more as compared to lambs in untreated group I (69.88) (Table 1). An antibody titre is a measurement of how much antibody an organism has produced that recognizes a particular epitope. In lambs Stresomix supplementation had significantly (P<0.01) boost the antibody titre. The increase in antibody titre on Stresomix supplementation was because of its constituent herbs viz Magnifera indica [11], Ocimum sanctum [12,13], Withania somnifera [14] and Phyllanthus emblica [15] possess antistress and immuno modulatory activities.

Table 1. Competitive Elisa antibody titre in deccani lambs vaccinated with inactivated multivalent blue tongue vaccine

| Lamb no. | Control 0 day titre | Control 28th day titre | Stresomix 0 day titre | Stresomix 28th day titre |
|----------|---------------------|------------------------|----------------------|------------------------|
| 1        | 3.2                 | 69.1                   | 1                    | 2.2                    |
| 2        | 2.8                 | 69.4                   | 2                    | 2.9                    |
| 3        | 3.1                 | 71.2                   | 3                    | 3.1                    |
| 4        | 2.8                 | 70.6                   | 4                    | 2.3                    |
| 5        | 3.6                 | 68.2                   | 5                    | 3.8                    |
| 6        | 2.9                 | 70.8                   | 6                    | 2.9                    |
| Mean     | 3.07                | 69.88                  | Mean                 | 2.87                   |
| S.E      | 0.13                | 0.47                   | S.E                  | 0.24                   |

3.2 Antibody Titre in Deccani Adult Sheep

Similar result with significant (P<0.01) increase in antibody titre was obtained in case of adult sheep (84.57) of group II supplemented with Stresomix in comparison of untreated control group I (57.88) (Table 2). Significant increase (P<0.01) in antibody titre in Stresomix supplemented sheep was because of its constituent herbs viz Withania somnifera, Ocimum sanctum and Phyllanthus emblica that possess antidepressant [16], antioxidant [17] and adaptogenic [18] activities. Due to synergism, polyherbal formulation confers some added benefits not available in single herbal formulation [19].

3.3 Serum Biochemical Parameters

The biochemical indices monitored in the blood and liver are useful 'markers' for assessment of disease and to a reasonable extent the toxicity of the drug [20,21]. On Stresomix supplementation no significant change in serum biochemical parameters was observed (Table 3). In both the groups serum biochemical parameters varied non significantly. This shows that the Stresomix was safe during usage.

Table 2. Competitive Elisa antibody titre in deccani adult sheep vaccinated with inactivated multivalent blue tongue vaccine

| Lamb no. | Control 0 day titre | Control 28th day titre | Stresomix 0 day titre | Stresomix 28th day titre |
|----------|---------------------|------------------------|----------------------|------------------------|
| 1        | 22.8                | 58.1                   | 1                    | 13.7                   |
| 2        | 13.6                | 52.1                   | 2                    | 17.9                   |
| 3        | 16.8                | 65.4                   | 3                    | 21.0                   |
| 4        | 17.2                | 58.2                   | 4                    | 0.17                   |
| 5        | 9.6                 | 55.3                   | 5                    | 17.4                   |
| 6        | 11.2                | 58.2                   | 6                    | 18.9                   |
| Mean     | 15.20               | 57.88                  | Mean                 | 14.85                  |
| S.E      | 1.95                | 1.80                   | S.E                  | 3.09                   |

Table 3. Competitive Elisa antibody titre in deccani adult sheep vaccinated with inactivated multivalent blue tongue vaccine

| Lamb no. | Control 0 day titre | Control 28th day titre | Stresomix 0 day titre | Stresomix 28th day titre |
|----------|---------------------|------------------------|----------------------|------------------------|
| 1        | 3.2                 | 69.1                   | 1                    | 2.2                    |
| 2        | 2.8                 | 69.4                   | 2                    | 2.9                    |
| 3        | 3.1                 | 71.2                   | 3                    | 3.1                    |
| 4        | 2.8                 | 70.6                   | 4                    | 2.3                    |
| 5        | 3.6                 | 68.2                   | 5                    | 3.8                    |
| 6        | 2.9                 | 70.8                   | 6                    | 2.9                    |
| Mean     | 3.07                | 69.88                  | Mean                 | 2.87                   |
| S.E      | 0.13                | 0.47                   | S.E                  | 0.24                   |
Table 3. Blood biochemical and liver function profiles in deccani sheep fed with or without herbal immunomodulators products

| Parameter               | Control | Stresomix |
|-------------------------|---------|-----------|
| **Blood biochemical profiles** |         |           |
| Serum albumen (g/dl)    | 3.46    | 3.50      |
| Blood urea nitrogen (mg/dl) | 12.68  | 12.71    |
| Creatinine (mg/dl)      | 1.36    | 1.38      |
| **Liver function tests** |         |           |
| SGOT (IU/dl)            | 43.22   | 43.26     |
| SGPT (IU/dl)            | 8.02    | 8.02      |

4. CONCLUSION

Stresomix efficiently potentiate the post vaccination antibody titre in sheep vaccinated with inactivated Blue tongue vaccine which means Stresomix, the polyherbal product can be recommended for its usage to improve post vaccination immune response in sheep.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Oluwatayo IB, Oluwatayo TB. Small ruminants as a source of financial security: A case study of women in rural Southwest Nigeria. IMTFI. 2012;2:1-21.
2. NCA. Report of the National Commission on Agriculture, Part VII: Animal Husbandry.
3. GOI. Report of the working group on animal husbandry and dairying for the eleventh five year plan (2007-2012). Government of India Planning Commission. New Delhi; 2007.
4. Sejian V, Maurya VP, Kumar K, Naqvi SMK. Effect of multiple stresses (Thermal, Nutritional, and Walking Stress) on the Reproductive Performance of Malpura Ewes. Vet Med Int. 2012;1(2012):1-5.
5. Saxena MJ, Madan P. Herbals for stress management in pets. The Vet. 1997;21(1):11-4.
6. Glick B, Day EJ, Thompson D. Calorie-protein deficiencies and the immune response of the chicken. I. Humoral Immunity. Poult Sci. 1981; 60:2494-2500.
7. Singh VK, Pattanaik AK, Goswami TK, Sharma K. Effect of varying the energy density of protein-adequate diets on nutrient metabolism, clinical chemistry, immune response and growth of Muzaffarnagari Lambs. Asian-Australas J Anim Sci. 2013;26(8):1089-1101.
8. Karakuş F. Weaning stress in lambs. J Int Sci Publ: Agric Food. 2014; 2:165-70.
9. Jochim MM. Improvement of the AGP test for blue-tongue Proc Annu Meet Am Assoc Vet Lab Diagn. 1976;19:361-76.
10. Snedecor GW, Cochran WG. Statistical methods, 8th ed. Oxford and IBH, Calcutta, India, 1994.
11. Makare N, Bodhankar S, Rangari V. Immunomodulatory activity of alcoholic extract of Mangifera indica L. in mice. J Ethnopharmacol. 2001;78(2-3):133-7. PMID: 11694357.
12. Mediratta PK, Sharma KK, Singh S. Evaluation of immunomodulatory potential of Ocimum sanctum seed oil and its possible mechanism of action. J Ethnopharmacol. 2002;80(1):15-20. PMID:11891082.
13. Sen P, Maiti PC, Puri S, Ray A, Audulov NA, Valdman AV. Mechanism of anti-stress activity of Ocimum sanctum Linn, eugenol and Tinospora malabarica in experimental animals. Indian J Exp Biol. 1992;30(7):592-6. PMID:1459632.
14. Muralikrishnan G, Dinda AK, Shakeel F. Immunomodulatory effects of Withania somnifera on azoxymethane induced experimental colon cancer in mice. Immunol Invest. 2010;39(7):688-98. PMID:20840055.
15. Liu X, Zhab M, Wu K, Chai X, Yu H, Tao Z, et al. Immunomodulatory and anticancer activities of phenolics from emblica fruit (Phyllanthus emblica L.). Food Chem. 2012;131(2): 685-690. DOI:10.1016/j.foodchem.2011.09.063.
16. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Anxiolytic-antidepressant activity of Withania somnifera glycowithanolides: An experimental study. Phytomedicine. 2000;7(6):463-9. PMID:11194174.
17. Kath RK, Gupta RK. Antioxidant activity of hydroalcoholic leaf extract of *Ocimum sanctum* in animal models of peptic ulcer. Indian J Physiol Pharmacol. 2006;50(4):391-6. PMID: 17402269.

18. Devi NKD, Shaheena S, Babu Ch D. Evaluation of adaptogenic activity of plant extracts by microorganisms. Der Pharmacia Lettre. 2014;6(4):184-89.

19. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of Ayurveda. Pharmacogn Rev. 2014;8(16):73-80.

20. Yakubu MT, Adesokan AA, Akanji MA. Biochemical changes in the Liver, Kidney and Serum of rat following chronic administration of cimetidine. African J Biomed Res. 2006;9:213-18.

21. Yakubu MT, Salau IO, Muhammad NO. Phosphatase activities in selected rat tissues following repeated administration of ranitidine. Nig J Biochem & Mol Biol. 2003;18(1):21-4.

© 2015 Ramana et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?id=1091&id=8&aid=9490