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Effect of *Saussurea lappa* (roots) and *Caesalpinia crista* (seeds), on Serum Protein Profile of *Fasciola* infected Buffaloes, in comparison with Triclabendazole

[*]ASMA WAHEED QURESHI[1], AKHTAR TANVEER [2], & NADEEM SHEIKH[2]

[1,] Department of Zoology, Abdul Wali Khan University Mardan, Pakistan.
[2,] Department of Zoology, University of the Punjab, Quaid-e-Azam campus, Lahore, Pakistan.

ABSTRACT

Serum protein profile study is useful to monitor changes in concentration and distribution of proteins (antigen/antibody). The present study was designed to analyze the biochemical effect of two medicinal plants i.e., *Saussurea lappa* and *Caesalpinia crista* on serum protein profiles of buffaloes, naturally infected with *Fasciola,* in comparison with Triclabendazole (an allopathic drug used against fasciolosis). Analysis of fractionized proteins before and after treatment revealed significant differences in the densities of serum proteins reflecting the changes at the transcriptional as well as translational level. In densitometric analysis, a total of nine peaks corresponding to nine different protein fractions i.e., 107, 98, 67, 56, 48, 43, 25, 19 and 15 kDa, were present before treatment in all infected samples. While in healthy control serum samples only eight peaks were found, which corresponded to peaks in infected sera except peak of 19kDa. After treatment densities of all fractions were almost to the level of healthy animal's sera. The protein fraction (19kDa) was either completely disappeared or significantly diffused, thereby indicating its association with fasciolosis in buffaloes. The nature of all the protein fractions observed in present study is needed a detailed investigation to identify them as antigen or antibody associated with fasciolosis.

Key Words: Fasciolosis, *Saussurea lappa*, *Caesalpinia crista,* triclabendazole, serum protein profile

INTRODUCTION

Fasciolosis is worldwide distributed parasitic disease which has great impact on human as well as animal development. Several species have been described for this disease within the genus *Fasciola*, but only two species, *Fasciola hepatica* and *F. gigantica*, are commonly recognized in animals and humans (Mas-Coma *et al.,* 2005).

Various drugs are used for treating fasciolosis but drug of choice to treat fasciolosis is now triclabendazole, a benzimidazole compound, which has been used effectively for both adult and immature flukes (Keiser *et al*., 2007; Barduagni *et al.,* 2008). However, the risk of appearance of resistance to triclabendazole cannot be ignored as reported by Moll *et al*. (2000). So there is always need to test new medicines against infectious agents.

Usually coprological examination is used to diagnose fasciolosis as eggs of the parasite can be observed in faeces. This diagnosis technique has many problems e.g., it gives positive results only after 3–4 months post infection. Very low egg shedding or even absence of egg was observed in case of presence of only one or a few adult flukes in host, (Esteban *et al.,* 1998; Mas-Coma *et al.,* 1999; Charlier *et al.,* 2008). So the serological diagnosis is preferred in which antigen-antibody reactions are involved and infection can be diagnosed much earlier (Charlier *et al.,* 2008).

Many researchers are working to obtain purified *Fasciola sp*. Excretory/secretory (E/S) antigens or recombinant molecules to improve serological tests. Cysteine proteinases are most abundant E/S products of *Fasciola*, which have shown to be a very valuable source of antigens for diagnosis. These highly antigenic enzymes secreted by the adult and juvenile and offer highly sensitive and specific markers for serodiagnosis for both *F. hepatica* and *F. gigantica* infection (Tantrawatpan *et al.,* 2005; El-Ridi *et al.,* 2007; Charlier *et al.,* 2008).

Serum protein profile study is useful to monitor changes in concentration and distribution of proteins. Furthermore, study on protein profile changes may serve as biomarkers for further investigation.

In present study post-treatment protein fraction changes in sera of infected buffaloes treated with different doses of two medicinal plants (*Saussurea lappa* and *Caesalpinia crista*) and triclabendazole (an allopathic medicine), were compared by SDS-PAGE, to identify the protein fractions associated with fasciolosis in buffaloes.

MATERIALS AND METHODS

Buffaloes (*Babulus sp.*) of Nili Ravi breed, naturally infected with fasciolosis were used for this

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study at Military dairy farm Barki road, Lahore, Pakistan (1 acre area). The age of animals were 6.0±1.0 years in both sexes. All these animals were tagged and kept under similar feeding and managemental conditions throughout the course of treatment and were given seasonal food and water *ad labitum.* A veterinarian was available for routine check up throughout the course of study.

Preparation of Herbal Drugs

Roots of *Saussurea lappa* and seeds of *Caesalpinia crista* were washed in tap water, then dried in oven at 40°C and crushed to powder. These powdered herbs were given in capsules orally, as described by Jhangir *et al.* (2003).

Experimental Design

A total of 62 infected animals (buffaloes) were randomly divided into 4 experimental and control groups i.e., C, D, T and F. All infected groups were compared with a group of 9 healthy animals (group G), which served as normal control. Groups C and D were further subdivided into three subgroups i.e., C1, C2, C3 and D1, D2, D3, respectively, each having 9 animals.

Buffaloes in sub-group C1, C2 and C3 were given separately, 50, 100 and 150mg/kg body weight *Saussurea lappa* and *s*ub-group D1, D2 and D3 were given, 50, 100 and 150mg/kg body weight *Caesalpinia crista*, respectively. Animals in Group T (having 9 infected buffaloes) were given 10mg/kg body weight Triclabendazole. Animals of group F (having 9 infected buffaloes) were served as negative control. Faecal samples of all experimental buffaloes were monitored for *Fasciola* eggs before treatment (on zero day) and after treatment till 28th day of treatment when >90% egg reduction was achieved in all groups.

Blood samples of all animals were collected at day zero (before treatment) and on 28th day (after treatment).

Serum Protein Profile Study

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique (Laemmeli, 1970) was used to analyze serum profile of all buffaloes. Blood sera were run on 12% gel and low molecular weight protein markers of 66 to 14.2 kDa (Sigma) were used as standards (Fig., 1, 2a PM).

Image Capture of Gels and Quantification for Protein Fractions

To identify and quantify the electrophoretically resolved protein fractions the gels were scanned and read on Gene Genius Gel Doc System and Image J software (version 1.41). Data was analyzed statistically by using Microsoft SPSS 10.0 (U.S.A) and $P<0.05$ was considered significant at CI 95%.

RESULTS

The two herbal medicines (*S. lappa* and *C. crista)* and triclabendazole, used in present study, were very effective against fasciolosis. In this article only serum profile changes (analyzed by SDS-PAGE technique) of control and treated animals were presented (Fig., 1, 2a & 2b).

Serum protein profile of Pre-treatment animals

A total of nine protein fractions were observed in serum of infected animals (Fig., 1I, 2bI). The peaks labelled numerically from 1 to 9 correspond to protein fractions of 107, 98, 67, 56, 48, 43, 25, 190 and 15 kDa, respectively. Protein fraction 19kDa, represented by peak 8 in densitometric analysis, was absent in healthy control subjects while all other protein fractions were same as in infected subjects (Fig., 2aG & F).

Protein fractions of 107, 98 and 67kDa showed conspicuous peaks, 56 and 48kDa diffused peaks and 43 to 15kDa (6 to 9 bands) were with prominent peaks in densitometric analysis of infected animals (Fig., 2bI). However, the density of these protein bands was less in sera of healthy animals except 56kDa fraction (peak 4) which was higher as revealed by densitometric analysis (Fig., 2aG).

Post-treatment changes in Serum profile of experimental animals

Post-treatment serum profile changes were found significant for both treatments, Triclabendazole and with *Saussurea lappa* and *Caesalpinia crista*. Analysis of post-treatment sera showed a significant ($P<0.05$) decline in the densities of peaks of all protein fractions (1-9) except peak 4 and 8. Peak 4 (56kDa) demonstrated a significant elevation where as peak 8 (19kDa) was either diminished or completely disappeared (Fig., 1, 2b C1-C3, D2-D3 and E). In group F (infected control), an opposite pattern of changes was observed in serum profiles. All protein fractions showed elevation except 56kDa, which showed decline (Fig., 2aF, 2bF).

DISCUSSION

Protein analysis is useful for monitoring physiological changes in cells or organisms. In normal serum protein profile, identification and quantification of protein fractions enable the identification of specific protein fractions in serum profile of infected individuals associated with cause

of disease (Legrain *et al.,* 2011; Kolarich *et al.,* 2012).

In present study, the pre and post-treatment serum protein profile changes in buffaloes infected with fasciolosis, were studied by SDS-PAGE. The effect of two medicinal plants i.e., *Saussurea lappa* and *Caesalpinia crista*, on serum profile was compared with the triclabendazole (an allopathic drug). Before treatment a total of nine protein fractions represented by nine peaks were observed in densitometric analysis in all infected serum samples while protein fraction of 19kDa represented by peak 8 was absent in healthy animals. Significant ($P<0.05$) changes were observed in the densities of all fractions before and after treatment. Almost all fractions were reached to normal level. The protein fractions of 43kDa present in normal and as well as infected animals and 19kDa present only in infected subjects, were either significantly diffused or completely disappeared after treatment with herbs as well as with triclabendazole. While in infected control group densities (%) of these fractions significantly increased, indicating their strong association with fasciolosis in buffaloes. These fractions may be antigens or antibodies. 60-66kDa protein fractions were reported as promising candidate for immunodiagnosis against fasciolosis (Krailis *et al.,* 1999; Ortiz *et al.,* 2000). Some workers reported 25-29kDa protein fractions as immunoreactive *Fasciola* antigen (Abdul-Rehman *et al.,* 1999; Attallah *et al.,* 2002; Mohhamed *et al.,* 2004) while Abdel-Rehman and Abdel-Mageed (2004) reported eight protein fractions i.e., 191, 178, 148, 118, 111, 101, 98.5 and 45kDa associated with fasciolosis. These fractions were different from the present study. The difference in these fractions may be due to difference in host species for immunoreactivity.

CONCLUSION

The present study revealed the association of 19kDa and 43kDa protein fraction with fasciolosis but the nature of all the protein fractions observed in present study is needed a detailed investigation to identify them as antigen or antibody associated with fasciolosis. Acute fasciolosis can lead to the onset of acute response resulting in the release of acute phase proteins by liver in blood. On the other hand fasciolosis infection can lead to the activation of immune system resulting in the antibody production by immune cells.

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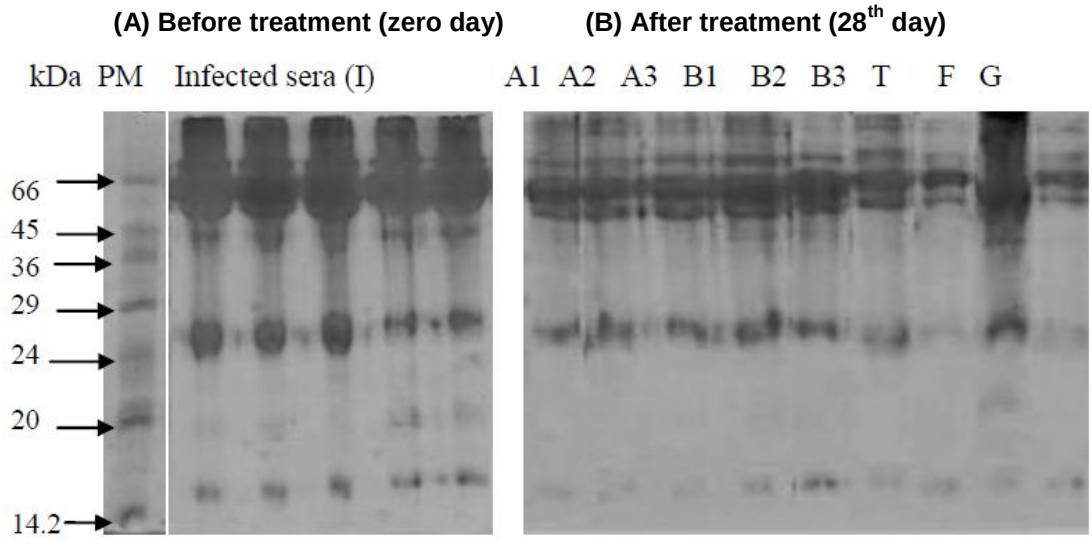

Fig., 1: Serum protein profile (SDS-PAGE) of buffaloes infected with fasciolosis: (A) before (I) and (B) after treatment (A1-G).

Buffaloes in Sub-group A1, A2 and A3 were given separately, 50, 100 and 150mg/kg body weight *Saussurea lappa*, Sub-Group B1, B2 and B3 *Caesalpinia crista*, respectively, on zero day and 18th day while buffaloes in Group T were given 10mg/kg body weight Triclabendazole only on zero day). Protein marker (PM), Infected Control (F), Healthy control (G), (n=9 per group/sub group).

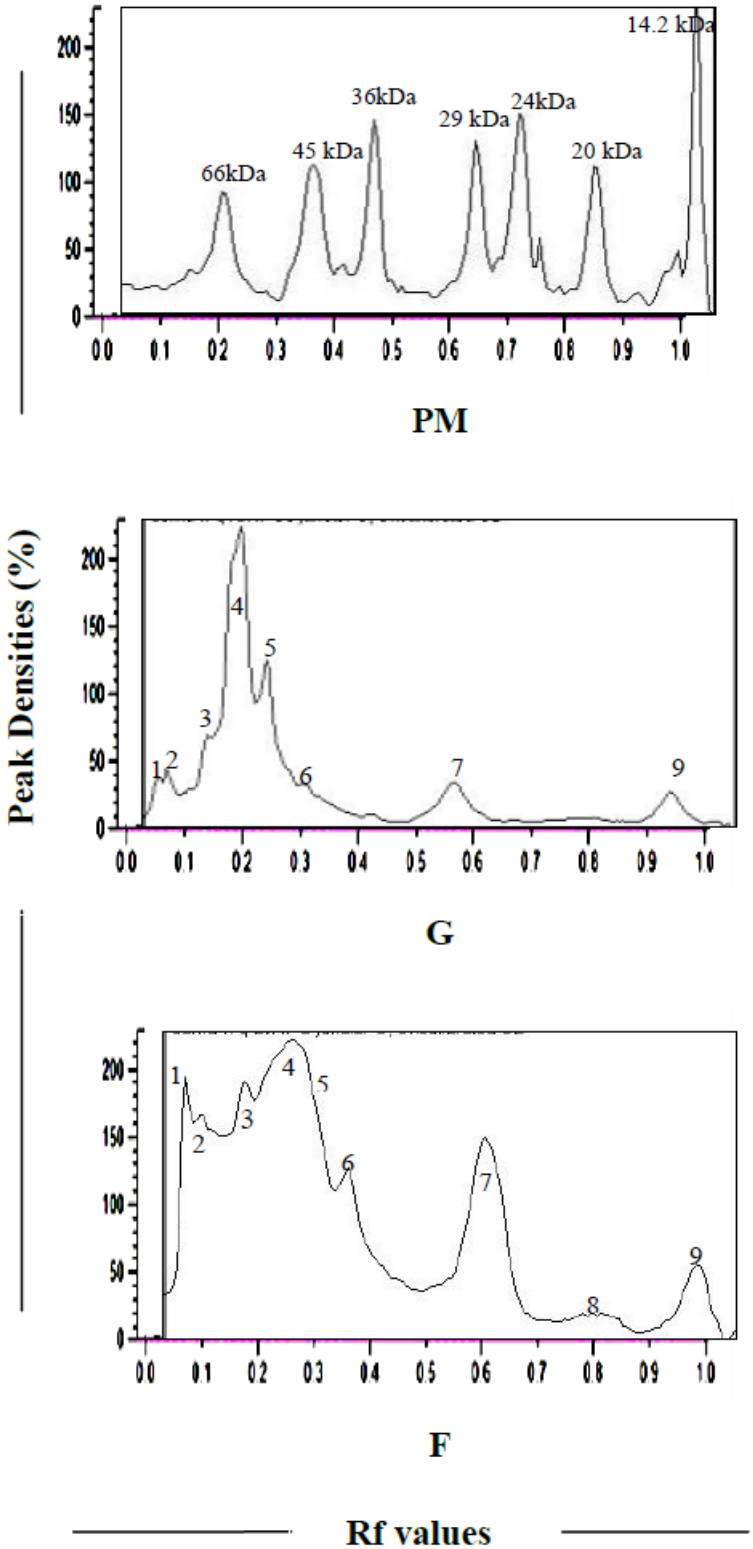

Fig., 2a: Densitometric curves showing protein profile of Protein marker (M), Healthy Control (G), Pre-treatment Infected Control (F).

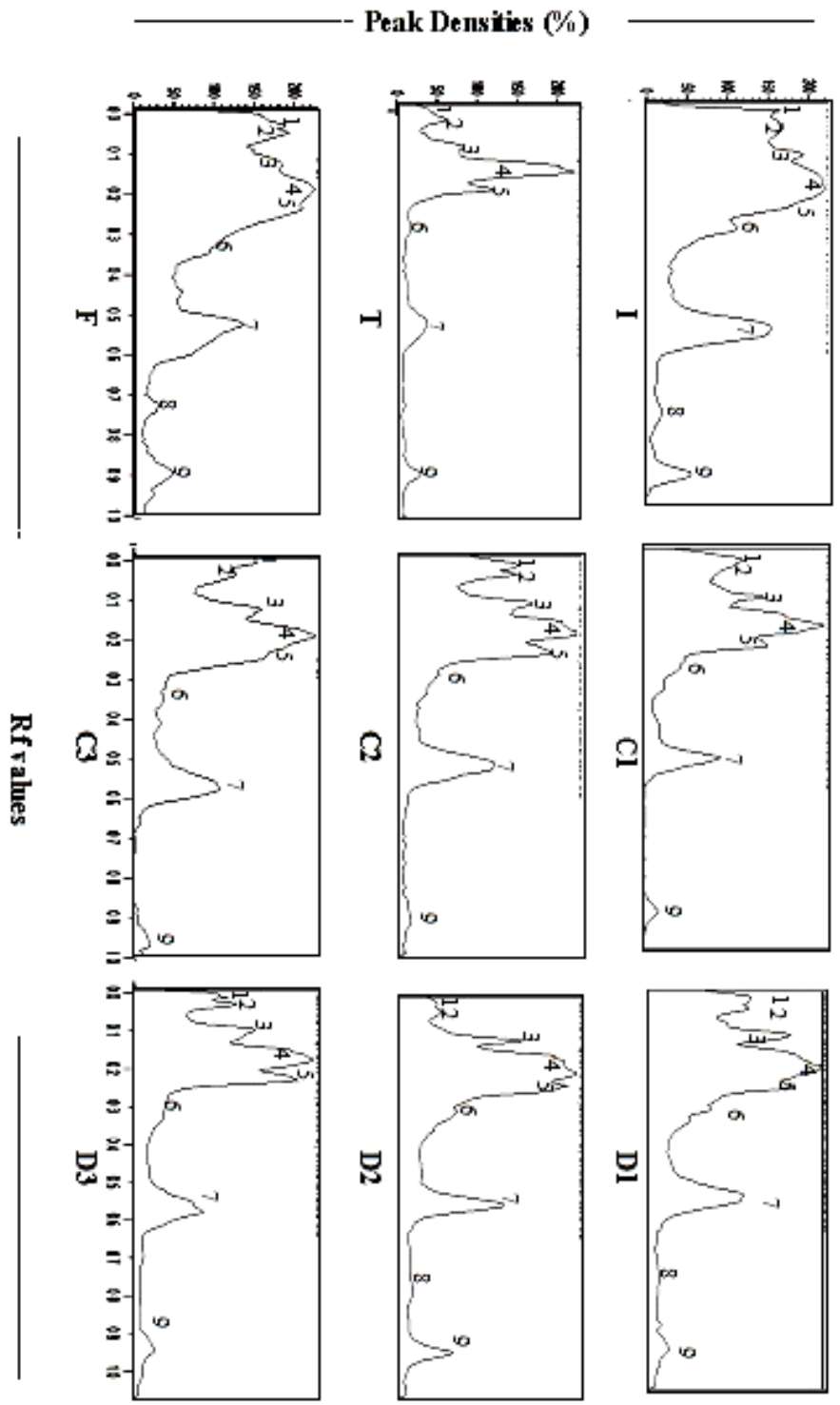

Fig., 2b: Densitometric curves showing protein profile of different groups of experimental animals: Pre-treatment Infected animal (I), Post-treatment: C1, C2 and C3 were given *Saussurea lappa* (50, 100 and 150mg/kg body weight, respectively), D1, D2 and D3 *Caesalpinia crista*, while Group T were given Triclabendazole (10mg/kg body weight), Infected Control (F). (n=9 per group/sub group). Peaks 1-9 representing the corresponding densities of protein fractions (107, 98, 67, 56, 48, 43, 25, 19 and 15 kDa, respectively).

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