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Biochemical Changes in the Livers of Bovines Naturally Infected with *Fasciola gigantica*

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Abstract: The present study was carried out to determine the total carbohydrate, total protein and total lipid content in the non-infected (Control) and naturally infected with *Fasciola gigantica* liver samples of buffaloes and cows. The estimated total protein and lipid content in the infected livers of these animals were found significantly higher as compared to their control samples. Total carbohydrate content in the infected livers of buffaloes and cows did not show any significant change as compared to their control animals.

Key words: *Fasciola gigantica*, biochemical changes, bovine liver, fascioliasis

INTRODUCTION

It is evident from the literature that fascioliasis is an economically important zoonotic disease, which mainly affects the livestock (Saleha, 1991; Daniel and Mitchell, 2002; Anonymous, 2003). *Fasciola hepatica* and *Fasciola gigantica* have been shown to be the causative agents of the disease in livestock (Smyth, 1994; Robert and Janovy, 2000). These flukes mainly attack the liver, where they reside and graze on the mucosa of the bile ducts and hepatic parenchyma resulting in the massive tissue damage (Shaikh *et al*., 2004, 2005). Evidence also suggests mortalities in bovines and ovines due to fascioliasis (Irfan, 1984; Losos, 1986).

Liver perform a number of essential functions in vertebrates (Young and Heath, 2001) and it is well known that liver damage has a variety of detrimental effects. It has already been documented that parasitic infection in liver and other tissues causes haematological, histopathological and biochemical changes in animals (Swarup and Pachauri, 1987; Mohsin *et al*., 1991; Lenton *et al*., 1996; Gill and Khan, 2004, 2005; Sharma *et al*., 2005; Kolodziejczyk *et al*., 2006).

Considering the importance of the livestock and *Fasciola gigantica* as a causative agent of the disease, current study was aimed to determine biochemical changes (Total carbohydrate, protein and lipid) that occur in the liver of bovines (buffaloes and cows) naturally infected with *Fasciola gigantica*.

MATERIALS AND METHODS

Collection and preparation of liver samples: Present investigation (2004) is based on the examination of the infected and non-infected livers of bovines (buffaloes and cows). Infected and non-infected liver samples were collected at random, by visual examination, from buffaloes and cows slaughtered at the main abattoir of Hyderabad city of Sindh province (Pakistan). These samples were immediately brought to the parasitology laboratory of Zoology Department, University of Sindh, Jamshoro, in ice box. These were then washed with normal saline, dried on blotting paper and chopped into small pieces for further studies. (In the text the term infected refers to those livers which harbor flukes whereas non-infected means control livers which do not harbor flukes and showed no gross anomalies.)

Collection and identification of liver flukes: The flukes were gently removed from the bile ducts of the infected livers of buffaloes and cows and thoroughly washed in saline solution prior to fixation. The specimens were fixed in AFA solution, then stained with Semichon's Acetic Carmine, cleared in xylene and finally mounted permanently in Canada balsam (Schmidt, 1988). The stained liver flukes were identified through the keys and description available in the literature (Soulsby, 1978; Yamaguti, 1971).

Biochemical studies: Infected and non-infected (Control) livers of buffaloes and cows were quantitatively analyzed for total protein, carbohydrate and lipid contents.

One gram of liver tissue was homogenized with distilled water by using tissue grinder (Pyrex brand). The homogenate was then centrifuged at 900 g for 15 min. Resulted supernatant was used for estimation of protein and carbohydrate contents. The technique of Lowry *et al*. (1951) was adopted for the quantitative assay for protein

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in the liver samples using Bovine Serum Albumin (BSA) as standard. The concentration of total carbohydrate was determined according to the method described by Dubois *et al.* (1956) with dextrin as standard. Total lipid content in the liver tissue was estimated following the method of Bligh and Dyer (1959). All experiments were conducted at 25±1°C.

Results of the total protein, carbohydrate and lipid content in the liver tissue are presented as milligram per gram of wet weight (mg g^{-1} ww).

Statistical analysis: Data obtained were subjected to student's t-test to determine the significant difference between non-infected (Control) and infected livers (Baily, 1981). Statistical significance was accepted at $p \leq 0.05$. Data were expressed as Mean±SEM.

RESULTS

Liver flukes recovered from buffaloes and cows were identified as *Fasciola gigantica.* Infected and non-infected livers of buffaloes and cows were analyzed for biochemical indices (Total protein, carbohydrate and lipid contents) and results are presented in Table 1 and 2.

The value for the protein content in control liver of buffaloes was 189.9±1.15. This value significantly ($p<0.001$) enhanced to 272.92±2.36 in liver samples of *Fasciola gigantica* infected buffaloes indicating an increase of 43.71% when compared with control ones. On the other hand, total lipid content in the liver of buffaloes significantly ($p<0.001$) increased from 50.34±1.00 to 61.64±3.17 in the infected buffaloes. Thus an elevation of 22.44% in the total lipid content was observed. The value (75.06±.41) of total carbohydrate content of the infected livers of buffaloes was not significantly different from the control ones (75.41±1.19).

A significant ($p<0.001$) rise in the value of total protein content of liver of cows from 162.58±2.05 (control) to 292.78±3.21 (infected), showing 80.08% more as compared to the control values, was noticed. Total lipid content of the liver also enhanced significantly ($p<0.001$) from control value of 37.31±0.84 to 46.40±1.27 in the infected cows indicating an elevation of 24.36%. However, the values for the total carbohydrate content in the infected (60.69±1.21) and control (60.55±0.98) liver of cows did not differ significantly.

Table 1: Biochemical indices of livers of control (non-infected) and infected buffaloes

Parameters	Total protein content (mg g^{-1} ww)	Total carbohydrate content (mg g^{-1} ww)	Total lipid content (mg g^{-1} ww)
Control (n = 10)	189.9±1.15	75.41±1.19	50.34±1.0
Infected (n = 10)	272.92±2.36*	75.06±1.41	61.64±3.17*
Change (%)	+43.71	-0.46	+22.44

*Significant: $p \leq 0.001$; + Increased, - Decreased

Table 2: Biochemical indices of livers of control (non-infected) and infected cows

Parameters	Total protein content (mg g^{-1} ww)	Total carbohydrate content (mg g^{-1} ww)	Total lipid content (mg g^{-1} ww)
Control (n = 10)	162.58±2.05	60.55±0.98	37.31±0.84
Infected (n = 10)	292.78±3.21*	60.69±1.21	46.40±1.27*
Change (%)	+80.08	-0.23	+24.36

*Significant: $p \leq 0.001$; + Increased, - Decreased

DISCUSSION

Fasciola hepatica and *Fasciola gigantica* induce their pathogenic effect on the host by causing the massive damage to the liver tissue (Isseroff *et al.*, 1977; Shaikh *et al.*, 2004, 2005). These damages definitely affect the biochemical parameters of the liver (Swarup and Pachauri, 1987; Lenton *et al.*, 1996; Sharma *et al.*, 2005). Present study revealed significant increase in the protein and lipid concentration in the infected livers as compared to the normal. However no change could be demonstrated in the carbohydrate contents in the liver samples of infected and control buffaloes and cows.

The adult trematodes are not suspected to deprive their hosts of nutrients except some vitamin and trace elements (Cheng, 1986). Few studies have been done to observe the variations in protein synthesis, lipid and carbohydrate metabolism in the livers of rat and lamb during fascioliasis (Bercheva, 1981; Galtier *et al.*, 1983, 1986). However, Swarup and Pachauri (1987) reported some significant biochemical changes in the livers infected with *Fasciola gigantica,* but their study was limited to the buffaloes only.

When compared with the control liver samples, a significant ($p<0.001$) increase of 43.71 and 80.08% was noticed in the values of total protein content of the *Fasciola gigantica* infected livers of buffaloes and cows, respectively. Swarup and Pachuri (1987) also reported similar observations on the protein content in the liver of buffaloes infected with *F. gigantica.* Such a high level of protein content in the infected liver of buffaloes was attributed to the marked fibrotic reactions (Swarup and Pachuri, 1987). However, Galtier *et al.* (1983, 1986) have reported no change in hepatic protein in the infected rat and sheep during fascioliasis.

Hepatic protein synthesis accounts for approximately 15% of the total body protein production (Burt and James, 1994). In tissue injury and inflammation, the increased production and secretion of some protein from hepatocytes, called acute phase response, is mediated by cytokines released by lymphocytes and macrophages (Burt and James, 1994). Information available showed that *Fasciola* synthesizes and releases large amount of proline

in the infected liver, which induced bile duct hyperplasia (Isseroff *et al*., 1977) and increased collagen contents (Modavi and Isseroff, 1984).

Elevated protein content recorded during present study might be due to the presence of proline and collagen. Acute phase response (Burt and James, 1994) and marked fibrotic reactions (Swarup and Pachuri, 1987) might have some contribution to it.

The liver is involved in many phases of lipid biotransformation and metabolism in ruminants. Lipids are the end products of anaerobic metabolism of carbohydrate in digenean flukes These flukes also excrete lipid droplets via excretory system as well as intestinal caeca (Harris and Cheng, 1973). The available information concerning the lipid content of the fluke infected liver of the rat showed decrease in total lipid concentration during second and forth week of post infection (Maffei-Facino *et al*., 1990, 1993; Lenton *et al*., 1995). Swarup and Pachauri (1987) have also reported reduced fat contents in the livers of buffaloes infected with *Fasciola gigantica.* However, an increase in fat has also been reported in rat liver with fascioliasis (Bercheva, 1981). In the present study significant rise in the values of total lipid content was 22.44 and 24.36% in the liver of buffaloes and cows respectively during *Fasciola gigantica* infection. The reason for high lipid content in the infected liver might be the accumulation of fat droplets or lipid dystrophy. During this study, no significant difference was seen in total carbohydrate contents in the infected and non-infected livers of buffaloes and cows. It is further mentioned that both buffaloes and cows indicated same pattern of biochemical effects induced by *Fasciola gigantica* infection. This observation suggests identical host response to *Fasciola gigantica* infection. The biochemical effects induced by the fluke in the liver are not direct one, but a histopathological manifestation.

Further study on biochemical aspect and metabolism of the liver during this disease in livestock is suggested, which will certainly help to elucidate the concept of host-parasite interaction.

REFERENCES

Anonymous, 2003. Fascioliasis causes losses in cattle, sheep and camelids. Vet. Rec., 152: 189-192.

Baily, N.T.J., 1981. Statistical Methods in Biology. 2nd Edn., Hodder and Stoughton, London, Sydney, Auckland. Toronto, pp: 43-51.

Bercheva, M., 1981. Changes in the liver lipids of rats with fascioliasis and in fascioliasis combined with protein-calorie malnutrition. Eksp. Med. Morphol., 20: 151-155.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method for total lipid and purification. Can. J. Biochem. Physiol., 37: 911-917.

Burt, A.D. and O.F.W. James, 1994. Pathophysiology of Liver. In: Pathology of Liver. Churchill Livingston. Macsween, R.N.M., P.A. Peter, J.S. Peter, D. Alistair, A.D. Burt and C.P. Brevard (Eds.), Edinburgh. London. Madrid. Melbourne. New York and Tokyo, pp: 57.

Cheng, T.C., 1986. General Parasitology. 2nd Edn., Academic Press. Inc., Orlando. Florida, pp: 369-371.

Daniel, R. and S. Mitchell, 2002. Fascioliasis in cattle and sheep. Vet. Rec., 151: 219.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebens and F. Smith, 1956. Calorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Galtier, P., A. Battaglia, J. More and M. Franc, 1983. Impairment of drug metabolism by the liver on experimental fascioliasis in the rat. J. Pharmacol., 35: 729-733.

Galtier, P., G. Larrieu, A. Tufelkji and M. France, 1986. Incidence of experimental fascioliasis on activity of drug metabolizing enzyme in lamb liver. Drug Metabol. Dispos., 14: 137-141.

Gill, N. and M.M. Khan, 2004. Effect of blood Protozoans on the haematological parameters of *Rattus norvegicus* of Hyderabad Sindh, Pakistan. Proc. Parasitol., 37: 17-25.

Gill, N. and M.M. Khan, 2005. Erythrocyte sedimentation rate in rats (*Rattus norvegicus*) naturally infected with endo-parasites. Pak. J. Zool., 37: 323-325.

Harris, K.R. and T.C. Cheng, 1973. Histochemical demonstration of fat associated with intestinal caeca of *Leucochloridiomorpha constantiae*. Trans. Am. Microsc. Soc., 92: 496-502.

Irfan, M., 1984. Pathology of the liver in fascioliasis in sheep. Pak. Vet. J., 4: 53-55.

Isseroff, H., P.R. Girard and M.D. Leve, 1977. *Fasciola hepatica*: Bile duct enlargement induced in rats after intra-peritoneal transplantation. Exp. Parasitol., 41: 405-409.

Kolodziejczyk, L., K. Siemieniuk and E. Skrzydlewska, 2006. *Fasciola hepatica*: Effects on the antioxidative properties and lipid peroxidation of rat serum. Exp. Parasitol., 113: 43-48.

Lenton, L.M., C.A. Behm and F.L. Bygrave, 1995. Aberrant mitochondrial respiration in the livers of rats infected with *Fasciola hepatica*: The role of elevated non-esterified fatty acids and altered phospholipids composition. Biochem. J., 307: 425-431.

Lenton, L.M., F.L. Bygrave, C.A. Behm and J.C. Boray, 1996. *Fasciola hepatica* infection in sheep: Changes in liver metabolism. Res. Vet. Sci., 61: 152-156.

Losos, G.J., 1986. Fascioliasis (*Fasciola gigantica*): In Infectious Tropical Diseases of Domestic Animals. 1st Edn., Churchill Livingstone Inc., New York, pp: 882-902.

Lowry, O.H., N.J. Rosebrough, A. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Bio. Chem., 193: 265-275.

Maffei-Facino, R., M. Carini, C. Genchi, O. Tofanetti, I. Casciarri and D. Bedoschi, 1990. Anti hepatotoxic properties of uridine diphosphoglucose in liver fluke infection. Arzneimittel-forschung/drug res., 40: 490-498.

Maffei-Facino, R., M. Carini, G. Aldini, R. Ceserani, I. Casciarri, E. Cavalletti and L. Verderio, 1993. Efficacy of glutathione for treatment of fascioliasis. An investigation in the experimentally infected rats. Arzneimittel-forschung/drug Res., 43: 455-460.

Modavi, S. and H. Isseroff, 1984. *Fasciola hepatica*: Collagen deposition and other histopathology in the rat host's bile duct caused by the parasite and by proline infusion. Exp. Parasitol., 58: 239-244.

Mohsin, M., M.M. Rehman, P.M. Das and A.K.M. Fazul Haque, 1991. Haematological observations in cattle naturally infected with *Fasciola gigantica*. Bangl. Vet., 8: 31-34.

Robert, L.S. and J. Janovy, 2000. Foundation of Parasitology. 6th Edn., J. McGraw Hill Book Co., pp: 256-259.

Saleha, A.A., 1991. Liver fluke disease (Fascioliasis): Epidemiology, economic impact and public health significance. Southeast Asian J. Trop. Med. Pub. Health, 22: 361-364.

Schmidt, G.D., 1988. Essentials of Parasitology. 4th Edn., Wm. C. Brown Publishers, Dubuque, Iowa, USA, pp. 243-252.

Shaikh, A.A., F.M. Bilqees, M.M. Khan, 2004. Bile duct hyperplasia and associated abnormalities in the buffaloes infected with *Fasciola gigantica*. Pak. J. Zool., 36: 231-237.

Shaikh, A.A., F.M. Bilqees, M.M. Khan, 2005. Histopathology of the liver of cow due to *Fasciola gigantica* infection. Proc. Parasitol., 40: 17-24.

Sharma, R.R., K.D. Prasad and A. Kumar, 2005. Haematobiochemical alteration in fascioliasis and its therapeutic management in cattle. Ind. Vet. J., 32: 997.

Smyth, J.D., 1994. Introduction to Animal Parasitology. 3rd Edn., Cambridge University Press. Cambridge, UK., pp: 205-211.

Soulsby, E.J.L., 1978. Helthminthes, Arthropods and Protozoa of Domesticated Animals. 6th Edn., Bailliere, Tindall and Cassell. Ltd., London, pp: 22-34.

Swarup, D. and S.P. Pachauri, 1987. Biochemical, histopathological and histochemical changes in liver of buffaloes affected by fascioliasis. Ind. J. Anim. Sci., 57: 1077-1082.

Young, B. and J.W. Heath, 2001. Weather's Functional Histology. A text and atlas. Churchill Livinstone Inc., pp: 274-281.

Yamaguti, S., 1971. Synopsis of Digenetic Trematodes of Vertebrates. Vol. I. Keigaku Publishing Co. Tokoyo. Japan, pp: 718-720.