The Bioscan 8(2): 459-462, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECT OF ANCYLOSTOMIASIS ON LIVER PROTEIN, AMINO ACIDS AND GST (GLUTATHIONE - S - TRANSFERASE) LEVEL IN MALE SWISS ALBINO MICE

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KEYWORDS
Ancylostomiasis
Liver
Protein
Amino acids
GST
Mice.

Received on :
29.10.2012

Accepted on :
14.02.2013

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ABSTRACT
The level of total protein, amino acids and GST (Glutathione –S- transferase) was estimated in the liver of male Swiss albino mice infected with various single doses (group A, 500 dose; group B, 1000 dose and group C, 2000 dose) of infective larvae of *Ancylostoma caninum*on day 1, 4, 9, 16 and 30 after infection. The level of liver protein was significantly increased in all the three groups (A, B and C) of mice when compared with control groups (a, b and c) on day 1, 4, 9, 16 and 30 of infection with a peak response on day 16 of infection (in all the three groups); in groups A, B and C, there was a sudden decline of liver protein on day 30 (still higher than control). Significant increase of amino acids and GST was found from day 1 to 30 in all the 3 groups of mice. The highest level of amino acids and GST was recorded on day 16 in mice of groups A and C and on day 9 in mice of group B. Increase of liver protein, amino acids and GST in infected mice indicates the occurrence of oxidative stress in hepatocytes due to infection which might be causing abnormality in energy metabolism.

INTRODUCTION

Ancylostoma caninum, the dog hookworm is present worldwide throughout most of the humid tropical and subtropical regions including India. Zoonotic infections can be defined as infections of animals that are naturally transmissible to humans. They are often spread in humans through their companion and domestic animals worldwide. Dogs and wild canids are definitive hosts of some intestinal parasites that cause important diseases in man and animals (Gholami *et al*., 2011). It is well known that *A. caninum* can cause human gut disease (Prociv and Croese, 1996; Prociv, 1998). Humans can get hookworm infection through ingestion of infective larvae or through direct penetration of the skin (Glickman and Schantz, 1981); the infective larvae penetrate the skin and induce symptoms of visceral larval migrans. The precise route of human infection by *A. caninum* larvae has not been established.Opportunities for percutaneous exposure abound in the endemic regions, where dog feces contaminate grass and soil and in people frequently walk bare foot, the possibility of oral exposure has never been seriously considered or investigated.

Transmission of *A. caninum* infective larvae to humans can occur by swallowing or inhaling pathogens from the reservoir hosts, eating the hosts or being bitten. Parasites may also be transmitted from animals to humans by vectors such as fleas, ticks and mosquitoes (Ostfeld and Holt, 2004). Human infection with larvae of *A. caninum* or *Toxocara canis* (canine parasites), cause infection and clinical manifestations, but the parasites do not complete their life cycle in the human host (Reinhard and Aufderheide, 1990).There have been reports on the migratory pattern of *A. caninum* larvae in oral and sub cutaneous infections in mice (Bhopale and Johri, 1975; Vardhani and Johri, 1981). It was found that about half of the orally administrated larvae migrate through the intestine into liver and lungs and to the musculature. In cutaneous infections, a substantial number of larvae were able to migrate to musculature directly without entering the visceral organs. Larval migration and distribution in the different tissues occur very early in mice infected with various multiple doses and more larvae were expelled from the gastrointestinal tract. Migration and persistence of *A. caninum* larvae in various tissues of vertebrates such as cat, mouse and monkey have been studied by various investigators (Kono and Sawada, 1961; Lee *et al*., 1975; Vardhani and Krishna Rao, 1995). There were some abnormal findings in liver of mice which were cutaneously infected with *A. caninum*. (Soh *et al*., 1969).

A. caninum is an important model for understanding the human hookworm infection and also as a zoonotic agent (Franke *et al*., 2011). The role played by liver in expelling and/ or destroying the infective *A. caninum* larvae and in controlling the severity of ancylostomiasis has documented by Viveka Vardhani and Shakunthala (2011). The pathophy siological changes that occur in liver of infected mice have

received little attention. Hence, a new vista has been opened to investigate the level of protein, amino acids and GST in liver of male Swiss albino mice infected with infective *A. caninum* larvae.

MATERIALS AND METHODS

Infective *A. caninum* larvae were cultured following the method of Sen *et al*. (1965). Six weeks old male Swiss albino mice (25-31g) were employed in this work. Three experiments were conducted in the present study. Ten mice were infected orally with a single dose *i.e.,* 10 mice with 500 larvae/mouse (group A), 10 mice with 1000 larvae/mouse (group B) and 10 mice with 2000 larvae/mouse (group C). Another three corresponding groups (a, b and c) (10 mice in each) were kept as uninfected controls for comparison. Two mice from each of the experimental and control groups were necropsied on day 1, 4, 9, 16 and 30 of experimental period. Tissues of liver were separated from normal and experimental groups of mice on day 1, 4, 9, 16 and 30 of infection and utilised for quantitative estimation of total protein, amino acids and GST following the method of Lowry *et al*. (1951), Moore and Stein (1948) and Habig *et al*. (1974) respectively.

RESULTS

Results are shown in Tables 1 to 3

Protein content in group A

The level of protein manifested in the low dose treated group (group A) was higher than the control value throughout the experimental period. The level of protein showed increase in an ascending fashion from day 1 to 16 but declined on day 30 (237.5μg/mg) - it was still higher than controls (189.08μg/mg). The level of protein was at peak on day 16 (291.71 μg/mg).

Amino acids content in group A

The level of amino acids found in experimental mice was found to be higher throughout the experimental tenure than the controls. In mice of group A, there is a standardized elevation of amino acids from day 1 to 30. A sudden rise was noticed on day 9 (806.0 μg/g) and further continued in an exponential burst of amino acids by day 16 (977.0 μg/g). The level of amino acids was at its pinnacle on day 16. But there is an abrupt fall of amino acids on the terminal day (30) of experiment (705.0 μg/g), but still it was higher than the normal values.

GST activity in group A

The activity of GST in mice infected with low dose showed higher value than the controls throughout the experimental period. The level of GST in test mice recorded on day 1 (82 units/mg protein) of infection was slightly higher than controls (79 units/mg protein). An accelerated increase of GST activity was vividly manifested from day 4 (85.5 units/mg protein) to 16 (96.5 units/mg protein). A slight depleted activity of GST was found on day 30 (89 units/mg protein). Nevertheless, the penache of GST activity holds on day 16.

Protein content in group B

Experimental mice of group B, (1000 larvae /mouse) showed enhanced protein values than the uninfected mice (group b) from day 1 to 30 of experimental period. Transcending and logarithmic rise of protein was found from day 1 (234.69 μg/mg) to 16 (295.06 μg/mg). A steep fall in protein level was observed on the terminal day of experimental tenure *i.e.,* day 30 (267.72 μg/mg), but it was still higher than controls. The level of protein in infected mice (group B) was at its epitome on day 16 (295.06 μg/mg) (the highest recorded value).

Amino acids content in group B

The level of amino acids found in mice of group B were higher than controls from day 1 to 30 of infection period. The level of amino acids showed a strategical enhancement from day 1 (685.5 μg/g) to 9 (815.0 μg/g). But a steep fall was found on day 16 (768.5 μg/g), which further continued till day 30 (703.5 μg/g). There are two important points of interest- the level of amino acids was higher and at its peak on day 9 (which is the initial phase of the experimental period) and the level of amino acids was lower on day 4 (720 μg/g) and higher on day 16 (768.5 μg/g).

GST activityin group B

The experimental mice of group B which were parasitized by medium dose (1000 L/mice) of *A. caninum* larvae showed heightened activity than the controls throughout the experimental period. The level of GST showed heavy rise on day 9 (122.5 μg/mg) and 16 (119 units/mg protein) with a peak response on day 9. But the GST activity slightly halted on the final day of experiment *i.e.,* day 30 (103 units/mg protein), but it is higher than control value.

Protein content in group C

Experimental mice of group C showed higher protein content in liver than the controls (group c) from day 1 to 30 of experimental period. The level of protein in experimental mice increased from day 1 to 16. But it is slightly receded by day 30; it was still above the normal value. An interesting facet noticed in experimental mice of group C was the protein level which stood nearly the same on day 9 (336.18 μg/mg) and 16 (336.10 μg/mg); this proves that the enigmatic correlance of protein metabolism in survivalence is in deep distress in cellular integrity cast by parasitic infections.

Amino acids content in group C

The level of amino acid in test mice of group C was found to be higher than the normals from day 1 to 30 of infection period. In case of group C, the amino acid level tend to rise in exponential manner from day 1 (704 μg/g) to 9 (853 μg/g). But it culminated in a steep declination on day 16 (809.05 μg/g) which further continued till day 30 (762.5 μg/g). The amino acids level in test mice was at the pinnacle in on day 9.

GST activity in group C

The activity of GST in infected mice (group C) showed higher values than the controls. The experimental mice showed gradual increase from day 1 (91.5 units/mg protein) to 16 (131 units/mg protein). There was a marked decrease by day 30 (102.5 units/mg protein) but it is still higher than controls and the recorded values of day 1 and 4 of infection. It is interesting to note that the pace of GST is found to be nearer on day 9 (129 units/mg protein) and 16 (131 units/mg protein).

Table 1: Protein (μg/mg) content in the liver of infected (groups A, B and C) and uninfected (groups a, b and c) mice at different days of experiment. Values are expressed in the mean derived from 5 observations

Days of Necropsy	Experimental groups A 500 larvae/mouse	B 1000 larvae/mouse	C 2000 larvae/mouse	Control groups A	b	c
1	200.67	234.69	279.69	189.09	189.09	189.09
4	222.37	260.75	299.67	189.07	189.06	189.08
9	263.68	280.64	336.18	189.05	189.09	189.06
16	291.71	295.06	336.1	189.03	189.07	189.01
30	237.5	267.72	315.31	189.08	189.04	189.04

Table 2: Amino acids (μg/g), content in the liver of infected (groups A, B and C) and uninfected (groups a, b and c) mice at different days of experiment. Values are expressed in the mean derived from 5 observations

Days of necropsy	Experimental groups A 500 larvae/mouse	B 1000 larvae/mouse	C 2000 larvae/mouse	Control groups A	b	c
1	612	685.5	704	595	595	595
4	681.5	720	789.5	596	596.5	596.5
9	806	815	853	595.5	595.5	595.5
16	977	768.5	809.5	596	596	596
30	705	703.5	762.5	595	595.5	595

Table 3: GST (units/mg protein) content in the liver of infected (groups A, B and C) and uninfected (groups a, b and c) mice at different days of experiment. Values are expressed in the mean derived from 5 observations

Days of necropsy	Experimental groups A 500 larvae/mouse	B 1000 larvae/mouse	C 2000 larvae/mouse	Control groups A	b	c
1	82	89	91.5	79	79	79.5
4	85.5	96.5	95	79.5	79.5	79.5
9	94.5	122.5	129	79	79	79
16	96.5	119	131	79	79.5	79.5
30	89	103	102.5	80	80	80

DISCUSSION

Liver plays a vital role in fighting infections, particularly those arising in the bowel. Hepatocytes play a significant role in synthesizing molecules that are utilized elsewhere to support homeostasis, in converting molecules of one type to another, and in regulating energy balances. Amino acid metabolism, urea synthesis and protein metabolism occur in the liver. Evidence of the defects in each of these parameters may be observed in hepatic diseases. These include abnormal plasma levels of amino acids, proteins, urea and ammonia.

Experimental mice which received a single dose of 500 (group A), 1000 (group B) and 2000 larvae (group C) showed remarkable changes in the quantitative values of protein, amino acids and GST. It is of interest to note that mice which received 500 (group A), 1000 (group B) and 2000 (group C) dose of larvae showed much variation in the biochemical assays (in the level of protein, amino acids and GST). Increased protein and decreased RNA synthesis were found in the liver of guinea pigs infected with *Coxiella burneti* (Mallavia and Paretsky, 1967; Thompson and Paretsky, 1973).

The increase of protein and amino acids in liver (in all the 3 experimental groups) suggests that the abnormal physiological changes were caused by the various single oral doses of infective larvae in the host system. It is clear that the liver has undergone stress due to the oral dose of infective larvae. Per Olof *et al*. (1984) reported increased protein and amino acid synthesis in liver of rats due to trauma and trauma complicated by sepsis. It is known that oxygen required for energy metabolism in aerobic organisms may generate reactive oxygen and nitrogen radicals that impair a wide variety of biological molecules like protein, DNA and lipid. The oxidative stress in liver is maximised due to the hepatocytes death and/or the abnormality in energy metabolism in liver as suggested by Inoue *et al*. (2003). The increase or decrease of protein might be due to the modulation in cell functions or damage in cellular constituents like proteins. Ames (1989), Stadtman (1992), Kasai (1997) and Inoue *et al*. (2003) also reported that in most of the mammalian tissues the Reactive Oxygen Species (ROS) react rapidly with a variety of molecules and thereby damaging the cellular constituents such as lipids, proteins and DNA. Schwizer (1996), Beckman and Ames (1997) also opined that large amount of reactive oxygen, nitrogen species generated by activated lymphocytes, inflammation and oxidative stress are the major causes that impair proteins and DNA in the tissues of mammals.

The occurrence of intensive proteolytic changes in the liver of singly inflected animals contributes to the elevation of amino acids. Sie (1985) also suggested that intensive proteolysis (due to pathogenic stress) may lead to the enhanced level of amino acids. Dukan and Nystrom (1999) suggested that reactive oxygen species/free radicals may react with proteins and/or DNA leading to their denaturation. Flagg *et al*. (1994) also suggested that Melondialdehyde (one of the end products of lipid preoxidation) reacts with primary amino groups of proteins and with nucleic acid bases of DNA, resulting in tissue damage

and breakage of DNA and RNA. Mice infected with *Plasmodium berghei* showed alterations in hepatic histology and phosphoprotein levels in mice (Maniam *et al*., 2012).

ACKNOWLEDGEMENTS

One of the authors (VVV) is thankful to UGC for providing financial assistance in the form of MRP. The author (GVK) is indebted to UGC for being a partial benefactor in carrying out the present investigations.

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