Effect on lipid profile in mice experimentally infected and vaccinated with *Aspicularis tetraptera*

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Abstract
The present study was aimed to find out the effect of *Aspicularis tetraptera* parasite (nematode) on lipid profile level (triglyceride, cholesterol, HDL and LDL) of mice infected and vaccinated with somatic antigen. The mice carrying heavy infection showed decrease in lipid profile values but after vaccination of somatic antigen, lipid profile values became normal.Experimental results reveal that egg somatic antigen are most effective.

Keywords
Aspicularis tetraptera, Triglyceride, Cholesterol, HDL, LDL, Lipid Profile.

Introduction

Helminths are multicellular parasitic organism having medical and economic value. Many human beings and domesticated animals were found infected worldwide by parasite of helminth. Interestingly it was reported that parasite (helminths) survive as adults for 1-2 years [1]. Helminths were key factor of morbidity and mortality, especially in developing countries [2]. The World Health Organisation report indicates that more than 2.5 billion human are found infected with parasitic worm [3]. Intestinal helminths infections are more in children [4]. An estimate indicate that by year 2025, approximate 57% of human population (countries developing) may be infected [5-6] by helminth infection. Helminth borne diseases are primary factor in lowering the productivity of useful animals. This was mainly due to mortality and reduced weight gains etc. [7-8]. This problem is severe in tropical countries where rains are good [9-10].The parasitic oxyurid nematode of the genera *Aspicularis* are detected routinely within rodent families. Despite of control measures the presence of rodent pinworm indicates deficiencies in the diagnostic and eradication process. Due to failures in identification of the presence of parasite, many institutions are not in position to; suggest effective treatment for the eradication of pinworms [11].The oxyurids (Pinworm), *Syphcia muris, Syphcia obvelata* and *Aspicularis tetraptera*, are common parasites which belong to laboratory rodents. The pinworms were seen to affect weight and growth and on one side create various disorders of the intestine on other side [12, 13, 14, 15, 16, 17].The gastrointestinal helminths became resistant to anthelminthic drugs when they were regularly used which create problem in treatment of helminthic diseases [18]. Looking to the importance of problem present study was undertaken to evaluate efficacy of somatic antigen on lipid profile level in infected and vaccinated mice.

Materials and Method

Experimental animals

The swiss Albino mice, *Mus musculusalbinus*was selected for the present study. These animals wereobtained from Pharmacy College, IPS Academy, Indore, and were maintained in the animal house along with normal condition of light, aeration and temperature. Only healthy, helminths infection free male mice of 7-8 weeks old and 30-50 gms were chosen as the experimental animals. It was confirmed by examining the stool of mice. Selected mice were kept in sterile cages and were given, water and balanced diet daily.

Maintenance of *Aspicularis tetraptera* strain:

Aspicularis tetraptera strain was originally obtained from Parasitology Laboratory of Zoology department, Govt. Holkar Science College, Indore (M.P). *Aspicularis tetraptera* is being routinely maintained in the laboratory by serial passage in healthy mice, after every 31st days post infection with a dose of 100 variable embryonated eggs. The infected animals provided the various stages of the parasite for experimental purposes. The methods employed for maintenance, infection and recovery stages of *Aspicularis tetraptera* was described by Wakelin [19].

Preparation and infection of eggs-inoculums:

a) First embryonated eggs were counted
b) Then dose was prepared by taking 100 eggs in 2.0 ml suspension
c) Dose was given orally by using feeding needle fitted syringe
d) After inoculation experimental mice were put in different cages as per experimental design
e) Mice placed in cages were fed with standard diet

Preparation of somatic antigens:

a) Process used include Homogenization and lyophilisation.

b) Eggs, larvae, adult stages were thoroughly washed with distilled water.

c) Eggs, larvae and adult were separately homogenised in culture medium which was free from protein.

d) Each homogenate were than lyophilised and stored at 4℃.

Blood collection and serum separation:

a) Experimental mice were dissected to expose heart.

b) Blood was collected from ventricle using 2ml glass syringe.

c) From syringe blood was transferred to 15 ml centrifuge tube.

d) These tube were kept in freezer.

e) In the tube clotting was observed.

f) From these tube serum was pipetted out and stored at 20℃.

Estimation of lipid profile

Estimation of plasma total cholesterol

The total cholesterol was determined by the enzymatic method of Allain.

Estimation of Plasma High Density Lipoprotein (HDL)

The HDL-Cholesterol was determined using redox test kit [20] the absorbance of the sample controlled and the standard respectively were read against the reagent blank using a spectrophotometer at 545nm

Estimation of Plasma Triglycerides

The plasma triglycerides were determined using glycerol phosphate oxidase – Peroxidase method [21] the intensity of coloured compound formed is measured at 545nm using a spectrophotometer.

Estimation of Plasma Low Density Lipoprotein (LDL)

The low density lipoprotein-cholesterol concentrate (LDL) was done by phosphor-tungstate magnesium chloride method.

Result

Lipid Profile values were taken on 31st day post infection from mice infected with *Aspicularis tetraptera* and vaccinated with different concentration of somatic antigen of egg, are summarised in tables (1, 2, and 3) and figures (1, 2 and 3).

Total Lipid (in mg/dl)

In the present study total lipid values579 mg/dl was observed in $NINVC_1$ and 438 mg/dl in $INVC_2$. The total lipid values in *A. tetraptera* infected and vaccinated mice with various concentration of somatic egg antigen were observed 465 mg/dl in $IVEgSoAg_1$, 510 mg/dl in $IVEgSoAg_2$, 540 mg/dl in $IVEgSoAg_3$, 545 mg/dl in $IVEgSoAg_4$ and 552 mg/dl in $IVEgSoAg_5$.

The total lipid values observed in *A. tetraptera* infected and vaccinated mice with various concentration of somatic antigen of Larvae were 450 mg/dl in $IVLSoAg_1$, 495 mg/dl in $IVLSoAg_2$, 510 mg/dl in $IVCSoAg_3$, 525 mg/dl in $IVLSoAg_4$ and 532 mg/dl in $IVLSoAg_5$.

The total lipid values observed in *A. tetraptera* infected and vaccinated mice with various concentration of somatic antigen of Adult worm were 440 mg/dl in $IVASoAg_1$, 482 mg/dl in $IVASoAg_2$, 498 mg/dl in $IVASoAg_3$, 505 mg/dl in $IVASoAg_4$, and 515 mg/dl in $IVASoAg_5$.

The total lipid values were found decreased in $INVC_2$ as compared to $NINVC_1$. But total lipid values were observed increased, when concentration of somatic antigen increased. The observed values were directly proportional to the concentration of somatic antigen (Tables 1, 2, and 3& figures 1, 2, and 3).

Cholesterol (In mg/dl)

The Cholesterol level was observed 123 mg/dl in $NINVC_1$ and 94 mg/dl in $INVC_2$. The cholesterol level in *A. tetraptera* infected and vaccinated mice with various concentration of egg somatic antigen were observed 101 mg/dl in $IVEgSoAg_1$, 109 mg/dl in $IVEgSoAg_2$, 116 mg/dl in $IVEgSoAg_3$, 119 mg/dl in $IVEgSoAg_4$ and 122 mg/dl in $IVEgSoAg_5$.

The cholesterol values in *A. tetraptera* infected and vaccinated with various concentration of larvae somatic antigen were observed 98 mg/dl in $IVLSoAg_1$, 108 mg/dl in $IVLSoAg_2$, 115 mg/dl in $IVLSoAg_3$, 116 mg/dl in $IVLSoAg_4$ and 120 mg/dl in $IVLSoAg_5$.

The cholesterol values in *Aspiacularis tetraptera* infected with various concentration of adult worm somatic antigen were observed 90 mg/dl in $IVASoAg_1$, 97 mg/dl in $IVASoAg_2$, 103 mg/dl in $IVASoAg_3$, 107 mg/dl in $IVASoAg_4$and 111 mg/dl in $IVASoAg_5$.

Cholesterol values found decreased in $INVC_2$ as compared to $NINVC_1$. The cholesterol level was observed increased when concentration of somatic antigen increased. The observed cholesterol values were directly proportional to the concentration of somatic antigen.

The maximum cholesterol was observed in the group $IVEgSoAg_5$ i.e. 122 mg/dl and minimum cholesterol i.e. 90 mg/dlwas observed in $IVASoAg_1$. The maximum cholesterol value shown by egg somatic antigen.

Low density lipoprotein (LDLin mg/dl)

The low density lipoprotein was 73.3 mg/dl in $NINVC_1$ and 51.45 mg/dl in $INVC_2$.

The low density lipoprotein (LDL) values in *A. tetraptera* infected and vaccinated mice with various concentration of egg somatic antigen were observed 58.4 mg/dl in $IVEgSoAg_1$, 60.2 mg/dl in $IVEgSoAg_2$, 65.5 mg/dl in $IVEgSoAg_3$, 66.4 mg/dl in $IVEgSoAg_4$, and 68.3 mg/dlin $IVEgSoAg_5$.

The low density lipoprotein values in *A. tetraptera* infected and vaccinated mice with various concentration of larvae somatic antigen were observed 54.3 mg/dl in $IVLSoAg_1$, 58.2 mg/dl in $IVLSoAg_2$, 60.2 mg/dl in $IVLSoAg_3$, 64.4 mg/dl in $IVLSoAg_4$ and 65.6 mg/dl in $IVLSoAg_5$.

The low density lipoprotein values in *A. tetraptera* infected and vaccinated mice with various concentration of adult worm somatic antigen wereobserved 45.2 mg/dl in $IVASoAg_1$, 48.4 mg/dl in $IVASoAg_2$, 52.8 mg/dl in $IVASoAg_3$, 56.4 mg/dl in $IVASoAg_4$ and 60.2 mg/dl in $IVASoAg_5$.

The LDL value was found decreased in $INVC_2$ as compared to $NINVC_1$. But LDL values were found increased as the concentration of somatic antigen increased. The values were directly proportional to the concentration of somatic antigen (tables1, 2, and 3and figures 1, 2, and 3).

The maximum LDL was observed in the group $IVEgSoAg_5$(68.3 mg/dl) and minimum value (45.2 mg/dl) in $IVASoAg_1$. The maximum LDL values were shown in egg somatic antigen.

High Density Lipoprotein (HDL in mg/dl)

The high density lipoprotein value was 36 mg/dl in $NINVC_1$ and 28.5 mg/dl in $INVC_2$.

The HDL value in *A. tetraptera* infected and vaccinated mice with various concentration of egg somatic antigen were observed 30 mg/dl in $IVEgSoAg_1$, 30 mg/dl in $IVEgSoAg_2$, 32 mg/dl in $IVEgSoAg_3$, 32 mg/dl in $IVEgSoAg_4$ and 34 mg/dl in $IVEgSoAg_5$.

The HDL value in *A. tetraptera* infected and vaccinated mice with various concentration of larvae somatic antigen were observed 29 mg/dl in $IVLSoAg_1$, 31 mg/dl in $IVLSoAg_2$, 31 mg/dl in $IVLSoAg_3$, 33 mg/dl in $IVLSoAg_4$ and 34 mg/dl in $IVLSoAg_5$.

The HDL value in *A. tetraptera* infected and vaccinated mice with various concentration of adult worm somatic antigen were observed 26 mg/dl in $IVASoAg_1$, 28 mg/dl in $IVASoAg_2$, 29 mg/dl in $IVASoAg_3$, 30 mg/dl in $IVASoAg_4$ and 30 mg/dl in$IVASoAg_5$.

The HDL values were found decreased in $INVC_2$ as compared to $NINVC_1$. But HDL values were found increased when the concentration of somatic antigen increased. The obtained HDL was directly proportional to the concentration of somatic antigen (tables 1, 2, and 3 and figures 1, 2, and 3).

The maximum High Density Lipoprotein was observed 34 mg/dl in $IVEgSoAg_5$and $IVLSoAg_5$and minimum HDL value 26 mg/dl in $IVASoAg_1$.

Table-1

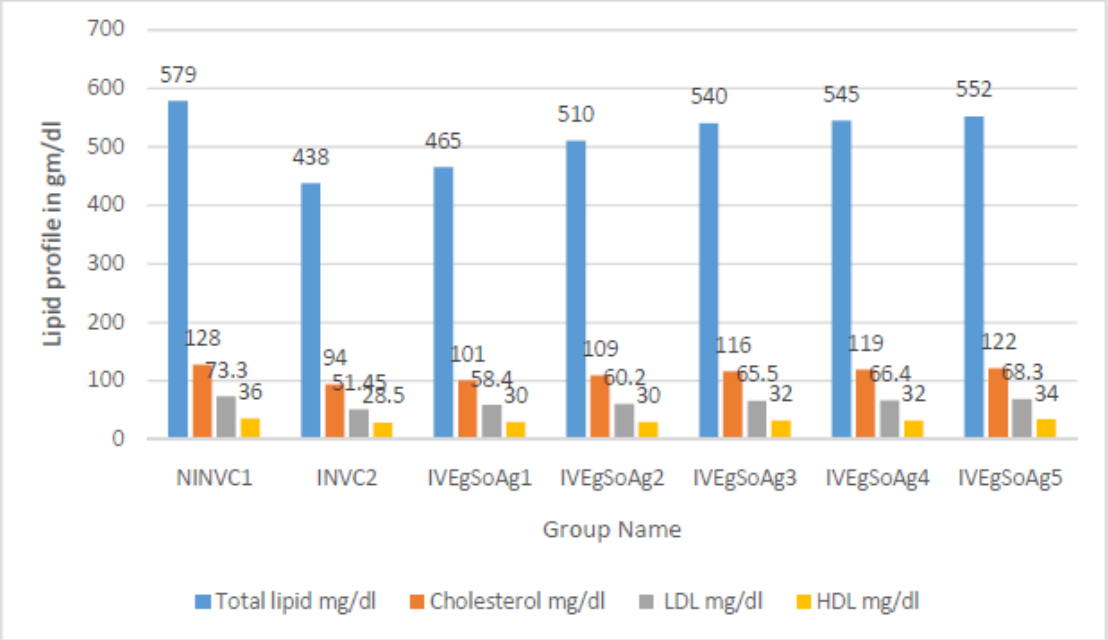
Lipid Profile values taken on 31st day post infection from mice infected with *A. tetraptera* and vaccinated with different concentrations of somatic antigen of eggs.

Group No.	Groups	Total lipid mg/dl	Cholesterol mg/dl	Low density lipoprotein (LDL) mg/dl	High density lipoprotein (HDL) mg/dl
1.	$NINVC_1$	579 ±3.834	128 ±2.280	73.3 ±0.698	36 ±2.828
2	$INVC_2$	438 ±4.295	94 ±2.828	51.45 ±0.664	28.5 ±1.612
3	$IVEgSoAg_1$	465 ±3.405	101 ±2.828	58.4 ±0.282	30 ±0.7071
4	$IVEgSoAg_2$	510 ±0.894	109 ±2.607	60.2 ±0.070	30 ±0.368
5	$IVEgSoAg_3$	540 ±1.414	116 ±3.847	65.5 ±3.479	32 ±1.581
6	$IVEgSoAg_4$	545 ±4.939	119 ±3.84	66.4 ±0.2	32 ±0.894
7	$IVEgSoAg_5$	552 ±1.414	122 ±1.414	68.3 ±0.2	34 ±1.414

NINVC$_1$	Non infected non vaccinated control-1
INVC$_2$	Infected non vaccinated control-2
IVEgSoAg$_1$	Infected vaccinated with 20µg eggs somatic antigen.
IVEgSoAg$_2$	Infected vaccinated with 40µg eggs somatic antigen.
IVEgSoAg$_3$	Infected vaccinated with 50µg eggs somatic antigen.
IVEgSoAg$_4$	Infected vaccinated with 80µg eggs somatic antigen.
IVEgSoAg$_5$	Infected vaccinated with 100µg eggs somatic antigen.

Figure-1

ShowingLipid Profile values taken on 31st day post infection from mice infected with *A. tetraptera* and vaccinated with different concentrations of somatic antigen of eggs.



$NINVC_1$	Non infected non vaccinated control-1
$INVC_2$	Infected non vaccinated control -2
$IVEgSoAg_1$	Infected vaccinated with 20µg eggs somatic antigen.
$IVEgSoAg_2$	Infected vaccinated with 40µg eggs somatic antigen.
$IVEgSoAg_3$	Infected vaccinated with 60µg eggs somatic antigen.
$IVEgSoAg_4$	Infected vaccinated with 80µg eggs somatic antigen.
$IVEgSoAg_5$	Infected vaccinated with 100µg eggs somatic antigen.

Table-2

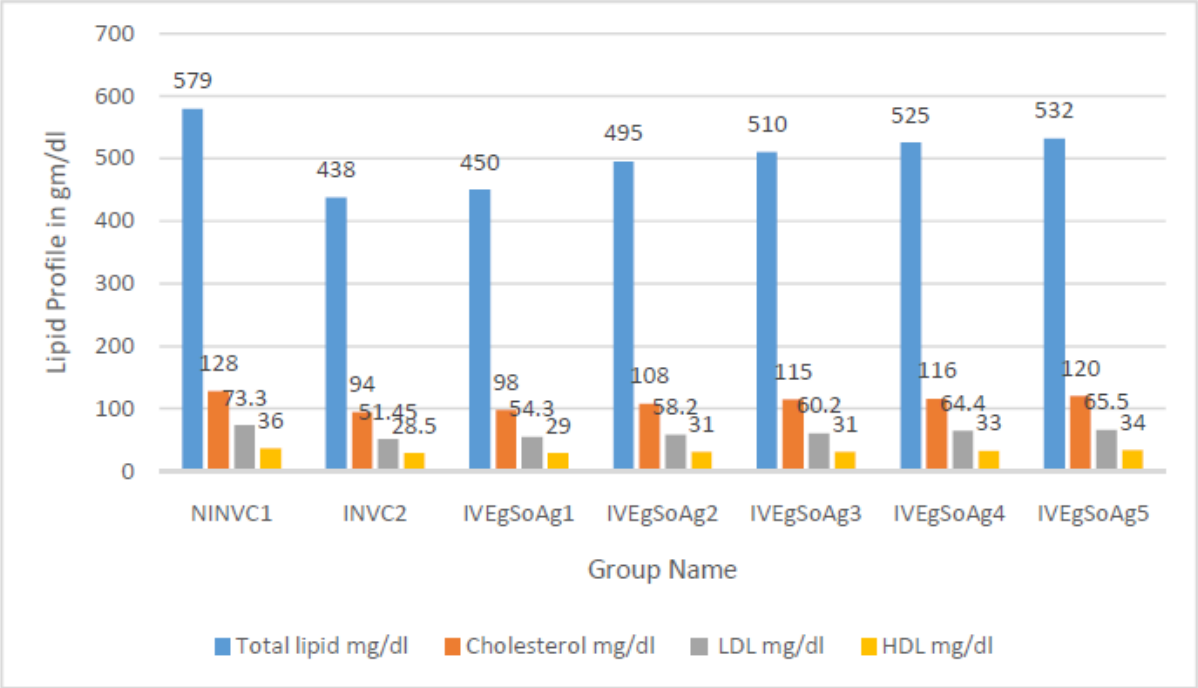
Lipid Profile values taken on 31st day post infection from mice infected with *A. tetraptera* and vaccinated with different concentrations of somatic antigen of larvae.

Group No.	Groups	Total lipid mg/dl	Cholesterol mg/dl	Low density lipoprotein (LDL) mg/dl	High density lipoprotein (HDL) mg/dl
1.	$NINVC_1$	579 ±3.405	128 ±1.414	73.3 ±0.698	36 ±2.366
2	$INVC_2$	438 ±4.73	94 ±2.828	51.45 ±0.664	28.5 ±1.673
3	$IVLSoAg_1$	450 ±3.405	98 ±1.414	54.3 ±0.384	29 ±0.824
4	$IVLSoAg_2$	495 ±3.687	108 ±4.472	58.2 ±0.469	31 ±3.847
5	$IVLSoAg_3$	510 ±1.266	115 ±2.607	60.2 ±3.289	31 ±1.414
6	$IVLSoAg_4$	525 ±0.282	116 ±3.687	64.4 ±0.316	33 ±1.41
7	$IVLSoAg_5$	532 ±0.635	120 ±2	65.5 ±0.352	34 ±3.687

$NINVC_1$	Non infected non vaccinated control-1
$INVC_2$	Infected non vaccinated control-2
$IVLSoAg_1$	Infected vaccinated with 20µg larvae somatic antigen.
$IVLSoAg_2$	Infected vaccinated with 40µg larvae somatic antigen.
$IVLSoAg_3$	Infected vaccinated with 50µg larvae somatic antigen.
$IVLSoAg_4$	Infected vaccinated with 80µg larvae somatic antigen.
$IVLSoAg_5$	Infected vaccinated with 100µg larvae somatic antigen.

Figure-2

ShowingLipid Profile values taken on 31st day post infection from mice infected with *A. tetraptera* and vaccinated with different concentrations of somatic antigen of larvae.



$NINVC_1$	Non infected non vaccinated control-1
$INVC_2$	Infected non vaccinated control -2
$IVEgSoAg_1$	Infected vaccinated with 20µg eggs somatic antigen.
$IVEgSoAg_2$	Infected vaccinated with 40µg eggs somatic antigen.
$IVEgSoAg_3$	Infected vaccinated with 60µg eggs somatic antigen.
$IVEgSoAg_4$	Infected vaccinated with 80µg eggs somatic antigen.
$IVEgSoAg_5$	Infected vaccinated with 100µg eggs somatic antigen.

Table-3

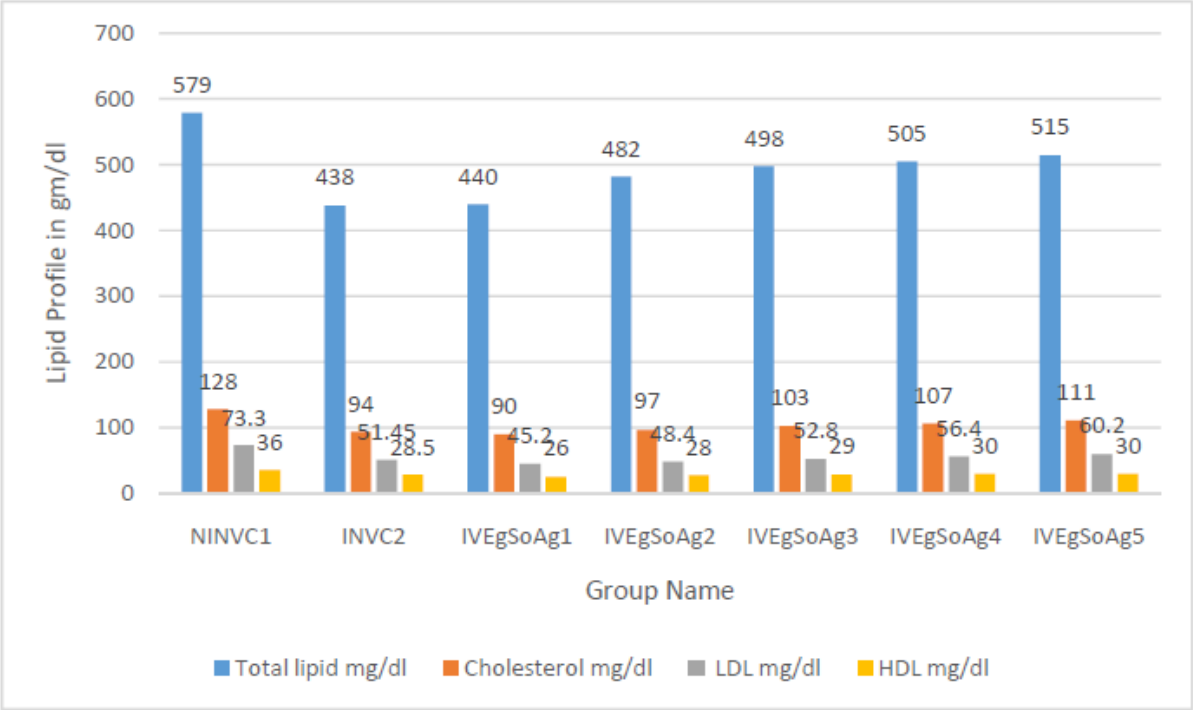
Lipid Profile values taken on 31st day post infection from mice infected with *A. tetraptera* and vaccinated with different concentrations of somatic antigen of antigen of adult worm.

Group No.	Groups	Total lipid mg/dl	Cholesterol mg/dl	Low density lipoprotein (LDL) mg/dl	High density lipoprotein (HDL) mg/dl
1.	$NINVC_1$	579 ±4.195	128 ±1.414	73.3 ±0.698	36 ±1.788
2	$INVC_2$	438 ±4.836	94 ±2.828	51.45 ±0.664	28.5 ±1.124
3	$IVLSoAg_1$	440 ±4.0496	90 ±3.224	45.2 ±0.322	26 ±3.224
4	$IVLSoAg_2$	482 ±1.266	97 ±0.920	48.4 ±0.316	28 ±0.322
5	$IVLSoAg_3$	498 ±0.644	103 ±2.828	52.8 ±0.572	29 ±0.141
6	$IVLSoAg_4$	505 ±2.607	107 ±2.607	56.4 ±2.151	30 ±1.279
7	$IVLSoAg_5$	515 ±0.6	111 ±2	60.2 ±0.447	30 ±0.850

$NINVC_1$	Non infected non vaccinated control-1
$INVC_2$	Infected non vaccinated control-2
$IVLSoAg_1$	Infected vaccinated with 20µg larvae somatic antigen.
$IVLSoAg_2$	Infected vaccinated with 40µg larvae somatic antigen.
$IVLSoAg_3$	Infected vaccinated with 50µg larvae somatic antigen.
$IVLSoAg_4$	Infected vaccinated with 80µg larvae somatic antigen.
$IVLSoAg_5$	Infected vaccinated with 100µg larvae somatic antigen.

Figure-3

ShowingLipid Profile values taken on 31st day post infection from mice infected with *A. tetraptera* and vaccinated with different concentrations of somatic antigen adult worm.



NINVC$_1$	Non infected non vaccinated control-1
INVC$_2$	Infected non vaccinated control -2
IVEgSoAg$_1$	Infected vaccinated with 20µg eggs somatic antigen.
IVEgSoAg$_2$	Infected vaccinated with 40µg eggs somatic antigen.
IVEgSoAg$_3$	Infected vaccinated with 60µg eggs somatic antigen.
IVEgSoAg$_4$	Infected vaccinated with 80µg eggs somatic antigen.
IVEgSoAg$_5$	Infected vaccinated with 100µg eggs somatic antigen.

Discussion

Results of lipid profile in infected and vaccinated mice with various concentration of somatic antigen are summarized in tables (1, 2 and 3) and presented in figures (1, 2 and 3). In the present study total lipids, cholesterol, LDL and HDL were found decreased in mice infected with *A. tetraptera*in comparison to control i.e. mice not infected with parasite. The decrease in lipid profile value was may be due to presence of parasite in gastrointestinal tract which utilized lipid content as food. But in infected and vaccinated mice (i.e. experimental mice treated with different concentration of somatic antigen) total lipid cholesterol, LDL and HDL were found increased may be due to killing and expulsion of parasites from the host. This confirm that vaccination (with somatic antigen) is effective in removing the parasite from the gastrointestinal tract of host. Results obtained suggest that infection caused decrease in the values of lipid profile. In my opinion this may be due to liver dis-function. Thus increase in value of lipid profile in the vaccinated mice may be the indication of recovery processes. Previous researchers also suggested that presence of parasite provoked hormone secretion and liver dis-function [22, 23]. Joshi [24] observed the decrease level of lipid content in liver of host infected by *B. malayi*. Wiedermann [25] also reported decreased serum lipid levels in the Shipibo population (Peru), which is infected with Hookworm, *Strongyloides* and *Trichuris*. Mohamed [26] also studied the total cholesterol, triglyceride, HDL, LDL and VLDL in camel infected with nematodes (*Strongyloides, Trichuris, Tichostrogylidae*). He also noted significant reduction in lipid values. According to these authors reduction in lipid profile may be due to the consumption of the lipid content form host body as a food by parasite.

Conclusion

In the present investigation Lipid profile values i.e. total lipid, cholesterol, LDL and HDL were found decreased in mice which were infected with *Aspicularis tetraptera,* but when experimental mice were vaccinated with somatic antigens of egg, larvae and adult worm (at various conc.), these values were seen remarkable increased.

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