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Lipid Profile Level of Nematode (*Aspicularis tetraptera*) Infected Mice, Treated with *Termanalia arjuna*.

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ABSTRACT

The present study was aimed to find out the effect of Nematode (*Aspicularis tetraptera*) parasite on lipid profile level (Triglyceride, Cholesterol, HDL and LDL) of mice infected and treated with Aqueous and Methanol extract of *Termanalia arjuna* bark. The mice carrying heavy infection showed decrease in the lipid profile level but after drug (Aqueous and Methanol extract of *Termanalia arjuna* bark) treatment lipid profile level was become on normal range due to killing of worm by drug. Methanol extract of *Termanalia arjuna* is more effective than aqueous extract of *Termanalia arjuna*.

Keywords: *Aspicularis tetraptera*, Triglyceride, Cholesterol, HDL, LDL, Lipid Profile and *Termanalia arjuna*.

INTRODUCTION

Helminths are recognized as a major constrain to livestock production throughout the world [1]. They are responsible for retarded growth [2], lowered productivity [3], mortality [4] and high economic losses [5]. The prevalence of helminths in different species of animals has been reported [6]. The prevalence of parasitic helminths typically displays a negative binomial distribution within an infected population such that relatively few persons carry heavy parasite burdens. Without treatment, those individuals are most likely to become ill and to perpetuate infection within their community [7]. Helminthes infections are among the most widespread infection in humans, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anemia and eosinophilia [8]. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas [9]. The gastrointestinal helminthes become resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases [10]. Hence there is an increasing demand towards natural anthelmintics. Therefore the present investigation was undertaken to evaluate the anthelmintic efficacy of *Termanalia arjuna* bark based on lipid profile level of infected and treated mice.

MATERIALS AND METHODS

Experimental Animal

The Inbred female Swiss albino mice, *Mus musculus albinus* of 6-8 weeks old and 15-20 gm in weight were selected as an experimental animals. Totally 60 mice were used. Five mice were used for positive control, 5 mice used for negative control and 50 mice used for experiment.

Experimental Parasite

For the present investigation *A. tetraptera* was selected as an experimental parasite and it being routinely maintained in the laboratory by serial passage.

Preparation of inoculums for infection

The 100 viable eggs were fed to each mouse. After inoculation, mice were kept in cages, labelled according to the design of experiments, were fed routinely with the same standard diet.

Test drug and Chemotherapy

Terminilia Arjuna plant (Bark) as a drug was used in the present experiments. 1 ml Aqueous and methanol extract of different concentration of drug was given to each mouse. The drug treatment was given on 17, 18 and 19th post infection days. Each dose was given once a day for three consecutive days (OD x 3). The different concentration of the proposed drug was administered in extract form to the infected mice to assess their therapeutic efficacy in experimental *Aspiculuris tetraptera* infected mice.

Extract preparation of drug in Aqueous

For this 0.1gm, 0.08 gm, 0.06 gm, 0.04 gm and 0.02 gm of plant part (Bark) was taken, dried and then powdered fine and 10 ml of distilled water was added respectively. It was then heated until it's become nearly half i.e. approximately 5 ml of each concentration solution, than it was filtered by what man filter paper no.1. Than it was centrifuged at 2000 rpm for 10 min. the supernatant which contain clear, fresh extract of respective part of medicinal plant was used for experimental work.

Extract preparation of drug in methanol

For this 0.1gm, 0.08 gm, 0.06 gm, 0.04 gm and 0.02 gm of plant part (Bark) was taken, dried and then powdered fine and 10 ml of methanol was added respectively. it was then filter with the help of whatman filter paper no.1. Filter solution was centrifuge at 5000 rpm for 10 minutes. Pellet was discarded and supernatant was used for experimental work.

Collection of the blood samples, separation of serum

Blood from experimental and control mice was collected by cardiac puncture under mild ether anesthesia, before incision each mouse were swabbed with 90% alcohol, heart exposed, blood collected from the ventricle by a 2 ml sterilized dry glass syringe. Than it was put in cold overnight for clotting after which serum carefully pipetted out in to clean sterilized serum collecting tubes and stored at -20^oC until required.

Estimation of lipid profile**Estimation of Plasma Total Cholesterol**

Total cholesterol concentration was determined by the enzymatic method of Allain [11].

Estimation of Plasma High Density Lipoprotein (HDL) – Cholesterol

The HDL- Cholesterol was determined using Randox test kit [12]. The absorbance of the samples, control 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 [13]. The intensity of the coloured compound formed is measured at 545nm using a spectrophotometer

Estimation of Plasma Low Density Lipoprotein (LDL) – Cholesterol

The low density lipoprotein – cholesterol concentration (LDL) was done by phosphor-tungstate magnesium chloride method [11].

RESULTS

The results of lipid profile level (Cholesterol, Triglyceride, HDL and LDL) in infected and treated mice summarized in table 1-2.

(A) Triglyceride Level

The Triglyceride level in NINTC-1 was 89 mg/dl and in INTC-2 was 74 mg/dl. The Triglyceride level was decrease in control-2 as compare to control-1.

When mice were treated with aqueous extract of *Termanilia arjuna* bark the triglyceride level were increased 74, 75, 79, 82 and 84 mg/dl at the doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml, 0.1gm/10ml respectively. So the maximum triglyceride level was 84 and minimum 74 mg/dl was found at the doses of 0.1gm/10ml and 0.02 gm/10ml respectively.

When infected mice were treated with aqueous extract of *Termanilia Arjuna* bark triglyceride level alter were found to be 0.0, 1.33, 6.32, 9.75 and 11.90% at the doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml respectively.

When mice were treated with methanol extract of *Termanilia arjuna* bark the triglyceride level were increased 76, 80, 84, 89 and 90 mg/dl at the doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml, and 0.1gm/10ml respectively. So the maximum triglyceride was 90 and minimum 76 mg/dl was found at the doses of 0.1gm/10ml & 0.02 gm/10ml respectively.

When infected mice were treated with methanol extract of *Termanilia arjuna* bark triglyceride level alter were found 2.63, 7.5, 11.90, 16.85 and 17.77% at the doses of 0.02gm/10ml, 0.04gm/ 10ml ,0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml respectively.

(B) Cholesterol Level

The cholesterol level in NINTC-1 was 128 mg/dl and in INTC-2 was 120 mg/dl. The cholesterol level was decrease in control-2 as compare to control-1.

When mice were treated with aqueous extract of *Termanilia arjuna* bark the Cholesterol level were increased 120, 120, 120, 122 and 124 mg/dl at the doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml, 0.1gm/10ml respectively. So the maximum cholesterol level was 124 and minimum 120 mg/dl was found at the doses of 0.1gm/10ml and 0.02 gm/10ml respectively.

When infected mice were treated with aqueous extract of *Termanilia Arjuna* bark the cholesterol level alter were found 0.0, 0.0, 0.0, 1.63 and 3.22% at the doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml respectively.

When mice was treated with methanol extract of *Termanilia arjuna* the cholesterol level were increased 120, 122, 123, 125, 127 mg/dl at doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml, and 0.1gm/10ml respectively. So the maximum cholesterol was 127 and minimum 120 mg/dl was found at the doses of 0.1gm/10ml & 0.02 gm/10ml respectively.

When infected mice was treated with methanol extract of *Termanilia arjuna* bark the cholesterol level alter were found 0.0, 0.0, 2.43, 4 and 5.51 per cent at the doses of 0.02gm/10ml, 0.04gm/10ml, 0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml respectively.

(C) HDL Level

The HDL level in NINTC-1 was 36 mg/dl and in INTC-2 was 33 mg/dl. The HDL level was decrease in control-2 as compare to control-1.

When mice were treated with aqueous extract of *Termanilia arjuna* bark the HDL level were 33, 33, 33, 34 and 34 mg/dl at the doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml respectively. So the maximum HDL level was 34 and minimum 33 mg/dl was found at the doses of 0.1gm/10ml and 0.02 gm/10ml respectively.

When infected mice were treated with aqueous extract of *Termanilia Arjuna* bark the HDL level alter were found 0.0, 0.0, 0.0, 2.94 and 2.94% at the doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml, 0.1gm/10ml respectively.

Table - 1: Lipid profile level in *A. tetraptera* infected mice treated with different concentrations of aqueous extract of *Termanalia arjuna* (Bark).

Group No.	Group name	Dose	Mean Value of Triglyceride in mg/dl	%Alter of Triglyceride	Mean Value of Cholesterol in mg/dl	%Alter of cholesterol	Mean Value of HDL in mg/dl	%Alter of HDL	Mean Value of LDL in mg/dl	%Alter of LDL
1.	NINTC ₁	-	89		128		36		73	
2.	INTC ₂	-	74		120		33		68	
3.	ITTAA ₁	0.02gm	74	0%	120	0%	33	0%	68	0%
4.	ITTAA ₂	0.04gm	75	1.33%	120	0%	33	0%	68	0%
5.	ITTAA ₃	0.06gm	79	6.32%	120	0%	33	0%	69	1.44%
6.	ITTAA ₄	0.08gm	82	9.75%	122	1.63%	34	2.94%	70	2.85%
7.	ITTAA ₅	0.1gm	84	11.90%	124	3.22%	34	2.94%	70	2.85%

Table -2: Lipid profile level in *A. tetraptera* infected mice treated with different concentrations of methanol extract of *Termanalia arjuna* (Bark).

Group No.	Group name	Dose	Mean Value of Triglyceride in mg/dl	% Alter of Triglyceride	Mean Value of Cholesterol in mg/dl	%Alter of Cholesterol	Mean Value of HDL in mg/dl	% Alter of HDL	Mean Value of LDL in mg/dl \pm S.D.	% Alter of LDL
1.	NINTC ₁	-	89		128		36		73	
2.	INTC ₂	-	74		120		33		68	
3.	ITTAM ₁	0.02gm	76	2.63%	120	0%	33	0%	70	2.85%
4.	ITTAM ₂	0.04gm	80	7.5%	122	1.63%	33	0%	72	5.55%
5.	ITTAM ₃	0.06gm	84	11.90%	123	2.43%	34	2.94%	72	5.55%
6.	ITTAM ₄	0.08gm	89	16.85%	125	4%	34	2.94%	74	8.10%
7.	ITTAM ₅	0.1gm	90	17.77%	127	5.51%	35	5.71%	74	8.10%

NINTC ₁	Non infected Non treated control-1
INTC ₂	Infected Non treated control-2.
ITTAA ₁	Infected treated with 0.02gm <i>Termanalia arjuna</i> aqueous extract.
ITTAA ₂	Infected treated with 0.04gm <i>Termanalia arjuna</i> aqueous extract.
ITTAA ₃	Infected treated with 0.06gm <i>Termanalia arjuna</i> aqueous extract.
ITTAA ₄	Infected treated with 0.08gm <i>Termanalia arjuna</i> aqueous extract.
ITTAA ₅	Infected treated with 0.1gm <i>Termanalia arjuna</i> aqueous extract.
ITTAM ₁	Infected treated with 0.02gm <i>Termanalia arjuna</i> methanol extract.
ITTAM ₂	Infected treated with 0.04gm <i>Termanalia arjuna</i> methanol extract.
ITTAM ₃	Infected treated with 0.06gm <i>Termanalia arjuna</i> methanol extract.
ITTAM ₄	Infected treated with 0.08gm <i>Termanalia arjuna</i> methanol extract.
ITTAM ₅	Infected treated with 0.1gm <i>Termanalia arjuna</i> methanol extract.
P.I.	Post infection

When mice was treated with Methanol extract of *Termanilia arjuna* bark the HDL level were increased 33, 33, 34, 34, 35 mg/dl at doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml respectively. So the maximum HDL was 35 and minimum 31mg/dl was found at the doses of 0.1gm/10ml & 0.02 gm/10ml respectively.

When infected mice was treated with Methanol extract of *Termanilia arjuna* bark HDL level alter were found 0.0, 0.0, 2.94, 2.94 and 5.71 % at the doses of 0.02gm/10ml, 0.04gm/10ml, 0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml respectively.

(D) LDL Level

The LDL level in NINTC-1 was 73 mg/dl and in INTC-2 was 68 mg/dl. The HDL level was decrease in control-2 as compare to control-1.

When mice were treated with aqueous extract of *Termanilia arjuna* bark the LDL level were increased 68, 68, 69, 70 and 70 mg/dl at the doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml

respectively. So the maximum LDL level was 70 and minimum 68 mg/dl was found at the doses of 0.1gm/10ml and 0.02 gm/10ml respectively.

When infected mice were treated with aqueous extract of *Termanilia Arjuna* bark LDL level alter were found 0, 0, 1.44, 2.85 and 2.85 % at the doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml respectively.

When mice was treated with methanol extract of *Termanilia arjuna* bark the LDL level were 70, 72, 72, 74 and 74 mg/dl at the doses of 0.02gm/10ml, 0.04 gm/10ml, 0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml respectively. So the maximum LDL was 74 and minimum 68mg/dl was found at the doses of 0.1gm/10ml & 0.02 gm/10ml respectively

When infected mice was treated with methanol extract of *Termanilia arjuna* bark LDL level alter were found 2.85, 5.55, 5.55, 8.10 and 8.10 % at the doses of 0.02gm/10ml, 0.04gm/10ml, 0.06gm/10ml, 0.08gm/10ml and 0.1gm/10ml respectively.

From the above mentioned results, we conclude that methanol extract of *Termanalia arjuna* bark is more effective than aqueous extract of *Termanilia arjuna* barkt.

DISCUSSION

Many authors observed the nematode infection cause the decrease in level of cholesterol, triglyceride, LDL and HDL. The changes are due to break liver function and presumably changes in hormones secretions which are provoked by the presence of parasite [14-20].

Wiedermann [21] observed the total cholesterol, triglyceride HDL and LDL level in nematode infected animal. They found significant reduction of cholesterol, triglyceride LDL and HDL. These finding suggest that parasite consume the lipid content of host body as a food. Woodruf [22] observed that the presence of *Ascaris* in children is often associated with poor nutritional states. The poor malnutrition may also account for the decreased plasma cholesterol. Biadun [23] reported decreased levels of total cholesterol (TC), high density lipoprotein – cholesterol (HDL – C), and triglycerides (TG) in guinea pigs.

CONCLUSION

In the present investigation total lipid level were found decreased, in mice infected with *A. tetraptera* as compared to non infected mice. This was due to parasite presents in the intestine of mice and parasites consume the lipid content as a food. Infected mice were treated with aqueous and methanol extract of *T. arjuna* lipid profile level were found increased. This was due to killing and expulsion of parasite by the drugs. Thus the present study results are supported by above mentioned authors. We concluded that intestinal helminthes (*A. tetraptera*) appear to decrease plasma lipids (cholesterol, triglyceride, LDL and HDL). The mechanisms involved in the interaction between the helminthes and the biochemical parameters need further investigation as these interactions have implications on the liver functioning and hormone secretion.

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