Discussion

*Haemonchus contortus* is an important abomasal helminth of small ruminants responsible for disease and major production losses worldwide. This blood-feeding parasite can cause severe anaemia and rapid death in afflicted livestock. Modern control programs for infection with the parasite have relied heavily on the use of commercial anthelmintics. However, extensive resistance of *H. contortus* to these drugs has developed and there is a need for alternative control strategies. One such strategy is to employ plant products that have natural anthelmintic properties.

The main object of this work was to demonstrate anthelmintic effect of green synthesized silver nanoparticles on some biochemical parameters of *H. contortus*. Mean percent egg hatch inhibition induced by green synthesized silver nanoparticles after 48h was 91 ± 1.76 percent with the highest concentration i.e. 2.0 ppm. Mean percent egg hatch inhibition induced by *Z. jujuba* leaf extract (Aqueous) after 48h is 25 ± 1.73 with lowest concentration (100 ppm) whereas 92 ± 2.08 percent caused by highest concentration (600 ppm). The synthetic compound albendazole induced 84% mortality at a very low concentration i.e. 0.5 ppm IC$_{50}$ and IC$_{90}$ value for Egg hatch Assay was 0.007 ppm and 7.713 ppm in case of Silver nanoparticles treatment and 301.837 ppm and 849.426 ppm in *Z. jujuba* leaf extract. Albendazole produced IC$_{50}$ at very low concentration i.e. 0.11 ppm.

*In vitro* anthelmintic study on adult *H. contortus* exhibited the highest concentration (30 ppm) induced 94 ± 3.3 percent adulticidal activity. Mean percent mortality induced by *Z. jujuba* leaf extract (aqueous) after 24h represented 30 ± 3.3 percent adulticidal activity at lowest concentration (500 ppm) whereas 93 ± 5.7 percent caused by highest concentration i.e. 1750 ppm. LC$_{50}$ and LC$_{90}$ value for *in vitro* effect on adult *H. contortus* was 15.30 ppm and 33.873 ppm in case of Silver nanoparticles treatment and 942.686 ppm and 2047.503 ppm in *Z. jujuba* leaf extract (aqueous). Albendazole produced LC$_{50}$ at low concentration i.e. 101.69 ppm.

A number of studies have been made to control *Haemonchus contortus* though egg hatch assay studies and on *in vitro* anthelmintic activity of various plant extracts by several workers (Min *et al.*, 2004; Shaik *et al.*, 2004; Lange *et al.*, 2006). However, the lethal concentrations of AgNPs shown here are very low in comparison to the previous workers.
(Githiori et al., 2004; Costa et al., 2006). Earlier, IC$_{50}$ of 0.001ppm for EHA and LC$_{50}$ of 11.26 ppm were reported against $H$. contortus by AgNPs synthesized from Neem leaf extract which were lower than the present values (Rahul, 2012). This may be attributed to the size of silver nanoparticles which was smaller in the earlier study (25-30 nm).

In the present study, the effect Z. jujuba aqueous extract as well as green synthesized AgNPs using this extract was also studied on the adult worms’ biochemical profile. The glycogen content decreased very significantly with increasing concentration of both biologically synthesized Silver nanoparticles and Z. jujuba leaf extract (Aqueous).

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Carbohydrates form the chief energy source in parasitic nematodes. The outstanding feature of carbohydrate breakdown in nematodes is the production of reduced organic end products and this occurs even under aerobic conditions. glycogen in most of the nematodes provides a significant reserve store of energy, particularly in forms which are in animals and which exist in environments of low oxygen tension (Sood, 2006). Amount of glycogen contents in $H$. contortus has been determined earlier. It contains considerable amount (8-12% fresh tissue of glycogen; Chopra and Premvati, 1979). Female has more glycogen than male (Premvati and Chopra, 1979). This is possibly related to the reproductive role of female rather than with general metabolism.

Carbohydrates form the major and possibly the sole energy source of parasitic nematodes. However, importance of lipids cannot be overlooked, these being structural and functional constituents. These are important components of membranes, which are in constant stage of dynamic equilibrium. Also, these are incorporated into eggs and are important energy reserves in the free-living stages of animal parasitic nematodes. Thus, it we are able to selectively inhibit Lipid biosynthesis in parasites, these would not be available for incorporation into eggs, and we can check the propagation, if not eliminate the parasite. The total lipids constitute 44mg/g of fresh tissue and 200 mg/g of dry tissue. In the present study similar amount of lipid content is observed i.e. 47.70 mg/g. However, significant amount of reduction (P<0.0001) was observed in lipid content in the treated with silver nanoparticles and Z. jujuba leaf extract.

A significant decrease was also recorded for protein content in treated worms in the present study. Parasitic worms are capable of efficient protein synthesis. Moreover they are
needed by the cells and living systems and enzymatic proteins carry out a cascade of metabolic activities for normal functioning of the system. This reduced protein content would disrupt the normal physiological activities of the worms and accounted for mortality at higher concentrations of AgNPs and leaf extracts.

In the present work, we found that the aqueous extract had substances with anthelmintic activity. Moreover, alkaloids and other terpenoids present in its aqueous extract contributed to the bioreduction of AgNO$_3$ to Ag$^+$ ions hence forming the silver nanoparticles which were characterized through TEM studies. This is first report where AgNPs are tested for their ovicidal and anthelmintic activity against *Haemonchus contortus* and affected biochemical profile adversely thus leading to the mortality of worms.