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Research Article

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Ocimum sanctum and *Mallotus philippinensis* as potential candidate for anthelmintic preparation

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ABSTRACT

Ex-vivo effects of methanol extracts of the leaves of Ocimum sanctum and fruit powder of Mallotus philippinensis were tested against phosphatases like alkaline phosphatase (ALPase), acid phosphatase (ACPase) and adenosine triphosphatase (ATPase) and glycolytic enzymes such as lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) of Moniezia expansa. Ex-vivo treatment of Moniezia expansa with plant extracts increased the activity of phosphatases whereas it decreased the activitity of glycolytic enzymes. The increased activity of phosphatases signifies damage to tissues whereas decreased activity of glycolytic enzymes may reduce the energy production of parasite. Both the extracts need further investigation for their anthelmintic activity.

Key words: *Ocimum sanctum, Mallotus philippinensis, Moniezia expansa*, phosphatases, glycolytic enzyme

INTRODUCTION

Helminth parasites infect livestock to a large extent and are crucial to veterinary health. Their impact on health reduces asset value through increased mortality, especially of young stock. In addition, helminth infections cause direct economic losses due to reduced production; yet another dimension is added by the fact that several helminth infections can be transferred to man (zoonosis) [1]. Control of helminthiasis has been centre of focus in biomedical research since time immemorial. Since, 1960's when first anthelmintic drugs for gastrointestinal parasite control became available, chemoprophylaxis has been the main method of deworming. Control is generally achieved by the use of synthetic anthelmintics in combination with grazing management. However, misuse and poor formulation of these products have led to development of anthelmintic resistance. Moreover these drugs are relatively expensive and often unavailable to farmers in rural areas. Internal parasites in ruminants constitute problem that returns periodically in all livestocks, therefore recourse of synthetic dewormers is only a short term solution. As a consequence of these problems and difficulties, parasitologists and small holder farmers have continued to use indigenous plants as livestock dewormers drawing upon centuries of traditional belief and use of Ethno veterinary medicines (EVM) [2]. A number of plants have been tested for their anthelmintic efficacy [3-7]. Some of the plants showing at least some anticestodal activity are garlic, Wildginger (*Asarum cannadensa*), seeds and latex of papaya (*Carica papaya*), Indian mulberry (*Morinda citrifolia*) fruit, extract of bark and root of *Ficus religiosa*, leaves of *Azadirachta indica*, leaves of *Ocimum sanctum* and fruit powder of *Mallotus philippinensis* [8].

In present research work the efficacy of anthelmintic active principle of *Ocimum sanctum* and *Mallotus philippinensis*, alteration in the activity of phosphatases and glycolytic enzymes in *Moniezia expansa* has been studied.

EXPERIMENTAL SECTION

Preparation of herbal extracts

Leaves of *Ocimum sanctum* and fruit powder of *Mallotus philippinensis* were collected, shade dried and grounded to powder. 50g of each powder was dissolved in 500ml of methanol and kept in oven at 60ºC for 24 hours, after filtration solvent was recovered by rotary evaporator at 40ºC, the extract was collected in a round bottom flask and stored in refrigerator at 4ºC.

Collection, processing and treatment of parasite

Live specimens of *Moniezia expansa* were collected from small intestine of goats/sheep in 0.9% phosphate buffer saline (PBS, pH 7.3) from freshly slaughtered hosts at local abattoir at Chandigarh (India). Parasites were washed 3-4times with PBS and treated with methanol extract 200mg/ml with appropriate amount of PBS, for one hour at room temperature. Parasites were divided into five groups. Group I served as untreated control, Group II was treated with Albendazole (0.05%), Group III with *Mallotus philippinensis* (M.P), Group IV with *Ocimum sanctum* (O.S), Group V with *O. sanctum* + *M. philippinensis* (O.S+M.P).

Homogenate preparation

After treatment parasites were again washed with PBS, blot dried and homogenised in PBS (20% w/v) using an electric homogenizer. Homogenate was then centrifuged at 2000 rpm (179 X g) for 30 minutes. Supernatant was stored in deep freezer (-20°C) until used for enzyme assays.

Enzyme Assays

Alkaline phosphatase (ALPase) and Acid phosphatase (ACPase) was assayed according to the method of Bessay [9]. Adenosine triphosphatase (ATPase) was assayed according to the method of Fiske [10].

Lactate dehydrogenase (LDH) was assayed according to the modified method of Bergmeyer [11]. Malate dehydrogenase (MDH) according to the method of Raval [12].

Protein estimation was done by the method of (Lowry *et al.* 1951) using bovine serum albumin (BSA) as the standard [13]. Specific activity of the enzymes is expressed as the units of enzyme activity per mg of protein.

Data collected from five groups were statistically analyzed and presented as means ± S.D. Comparisons of the mean values between treated and control was made using Student's t-test.

RESULTS AND DISCUSSION

The phosphatases are believed to be involved directly in the absorption of nutrients and it has been suggested that their presence may be indicative of active transport and is related to extracellular digestion [14-15]. ALPase and ACPase are the two vital enzymes of the tegumental and subtegumental regions in cestodes, with ALPase being the dominant enzyme [16-19]. ATPase, ALPase, ACPase are present in almost all structures of cestodes [20].

The present investigation reveals that there is increase in the activity of phosphatases (Table: 1), ACPase was increased by 45.45% (Albendazole), 51.51% (M.P), 60.60% (O.S) and 27.27% (O.S+M.P) as compared to untreated control (Figure 1). The specific activity of ALPase showed an increase of 13.13% (Albendazole), 38.66% (M.P), 42.66% (O.S) and 57.33% (O.S+M.P) (Figure 2). There is no significant alteration in the specific activity of ATPase, 2.67% (M.P), 0.31% (O.S), and 2.45% (O.S+M.P) except in the case of Albendazole treated parasites where an increase of 13.24% was observed (Figure 3). The increase in specific activity of phosphatases leads to tissue damage in parasites and hence may cause mortality. The increase of *Echinococcus multilocularis* alkaline phosphatase activity in culture supernatants during *in vitro* drug treatment with Albendazole derivatives correlates with the progressive degeneration and destruction of metacestode tissue [21].

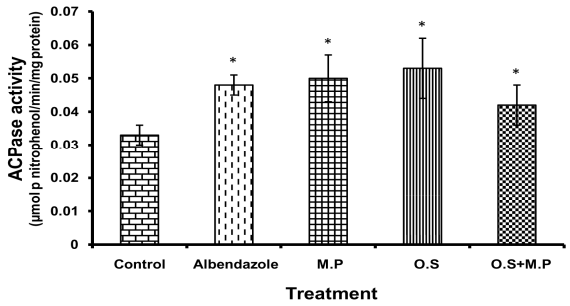

Fig. 1 Increase in specific activity of Acid phosphatase (ACPase) of *Moniezia expansa* treated with Albendazole and methanolic extracts of *Mallotus philippinensis* (M.P), *Ocimum sanctum* (O.S) and *Ocimum sanctum* + *Mallotus philippinensis* (O.S+M.P). Data is presented as Mean±S.D. Units ACP ; µmol p-nitrophenol /min/mg protein. Results are the average of 5 determinations, significant change as compared to control (*= p<0.05). Units are µmol p nitrophenol/min/mg protein

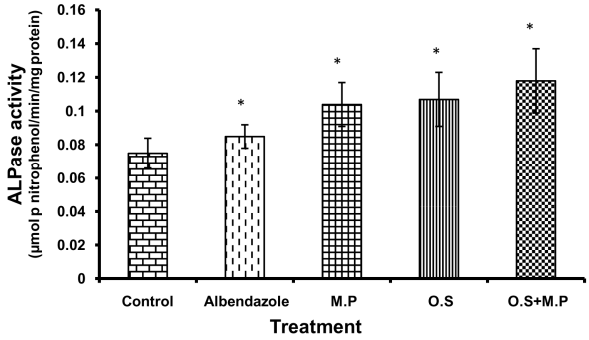

Fig. 2 Increase in specific activity of Alkaline phosphatase (ALPase) of *Moniezia expansa* treated with Albendazole and methanolic extracts of *Mallotus philippinensis* (M.P), *Ocimum sanctum* (O.S) and *Ocimum sanctum* + *Mallotus philippinensis* (O.S+M.P). Data is presented as Mean±S.D. Units ALPase ; µmol p-nitrophenol /min/mg protein. Results are the average of 5 determinations, significant change as compared to control (*= p<0.05). Units are µmol p-nitrophenol/min/mg protein

The glycolytic enzymes LDH and MDH are present in the cytosol of cell and play an important role in the carbohydrate metabolism of cestodes. In the present studies treatment with herbal extracts and Albendazole on *M. expansa* has been observed to bring about decrease in the activity of LDH by about 27.77% (Albendazole), 27.77% (M.P), 25% (O.S+M.P). No significant change was observed in parasite treated with O.S (Figure 4). Treatment with isatin has been reported to decrease the LDH activity in the metacestodes of *Echinococcus multilocularis*, in which glucose and glycogen stores also declined significantly [22]. The presence of MDH, which converts oxaloacetate to malate, has also been demonstrated in several cestodes. The decrease in the activity of MDH was 16.43% (M.P), 8.21% (O.S), and 32.87% (O.S+M.P). However 19.17% increase in specific activity was observed when treated with Albendazole (Fig: 5).

Table: 1 Effect of methanolic extracts of *Mallotus philippinensis* (M.P), *Ocimum sanctum* (O.S) and *Ocimum sanctum* + *Mallotus philippinensis* (O.S+M.P) and Albendazole on ACPase, ALPase, ATPase, LDH and MDH of *Moniezia expansa*

Group/Enzymes	ACPase (µmol pnitrophenol/min/mg protein)	ALPase (µmol pnitrophenol/min/mg protein)	ATPase (Pi/min/mg protein)	LDH (µmol NADH consumed/min/mg protein)	MDH (µmol NADH consumed/min/mg protein)
Control	0.033±0.003	0.075±0.009	5.452±0.534	0.036±0.003	0.073±0.006
Albendazole	0.048±0.003* (45.45%)	0.085±0.007* (13.33%)	6.174±0.070* (13.24%)	0.026±0.001* (27.77%)	0.087±0.008* (19.17%)
M. philippinensis (M.P)	0.050±0.007* (51.51%)	0.104±0.013* (38.66%)	5.598±0.356 (2.67%)	0.026±0.003* (27.77%)	0.061±0.008* (16.43%)
O. sanctum (O.S)	0.053±0.009* (60.60%)	0.107±0.016* (42.66%)	5.435±0.260 (0.31%)	0.035±0.005 (2.77%)	0.067±0.006* (8.21%)
O. sanctum + *M. philippinensis* (O.S+M.P)	0.042±0.006* (27.27%)	0.118±0.019* (57.33%)	5.586±0.299 (2.45%)	0.027±0.004* (25%)	0.049±0.007* (32.87%)

Data is presented as Mean ±S.D. (n=5) significant change as compared to control (= p<0.05).*

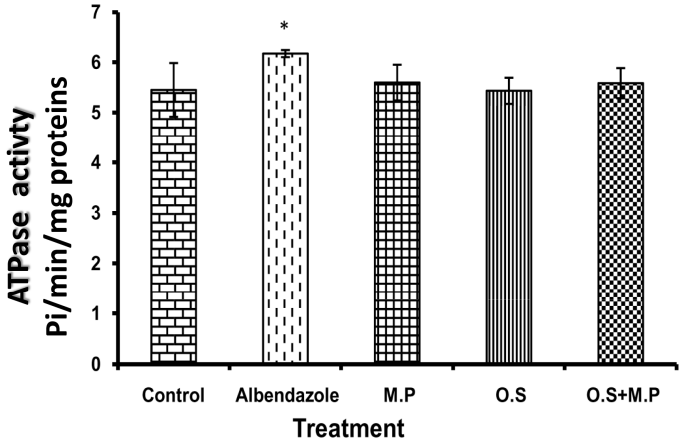

Fig. 3 Change in specific activity of Adenosine triphosphatase (ATPase)of *Moniezia expansa* treated with Albendazole and methanolic extracts of *Mallotus philippinensis* (M.P), *Ocimum sanctum* (O.S) and *Ocimum sanctum* + *Mallotus philippinensis* (O.S+M.P). Data is presented as Mean±S.D. Units ATPase ; Pi/min/mg protein. Results are the average of 5 determinations, significant change as compared to control (*= p<0.05).Units are µmol phosphate/min/mg protein

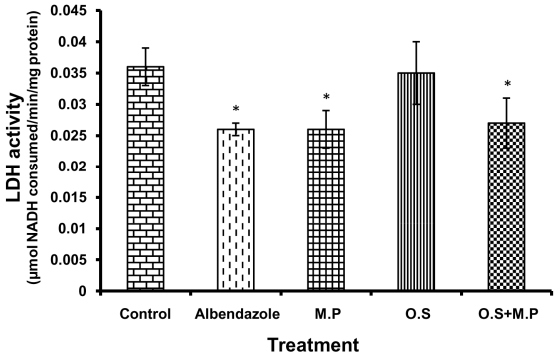

Fig. 4 Decrease in specific activity of Lactate dehydrogenase (LDH)of *Moniezia expansa* treated with Albendazole and methanolic extracts of *Mallotus philippinensis* (M.P), *Ocimum sanctum* (O.S) and *Ocimum sanctum* + *Mallotus philippinensis* (O.S+M.P). Data is presented as Mean±S.D. Units LDH ; µmol NADH consumed/min/mg protein. Results are the average of 5 determinations, significant change as compared to control (*= p<0.05). Units are µmol NADH consumed/min/mg protein

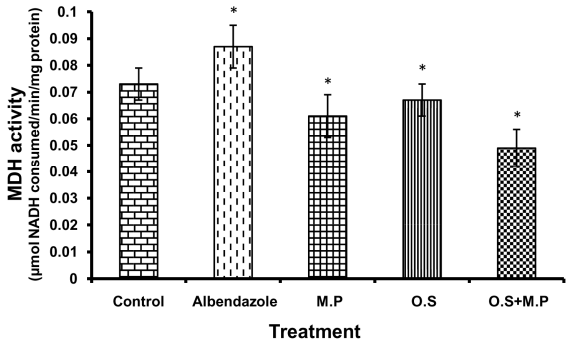

Fig. 5 Decrease in specific activity of Malate dehydrogenase (MDH)of *Moniezia expansa* treated with Albendazole and methanolic extracts of *Mallotus philippinensis* (M.P), *Ocimum sanctum* (O.S) and *Ocimum sanctum* + *Mallotus philippinensis* (O.S+M.P). Data is presented as Mean±S.D. Units MDH ; µmol NADH consumed/min/mg protein. Results are the average of 5 determinations, significant change as compared to control (*= p<0.05). Units are µmol NADH consumed/min/mg protein

The increase in activity of ACPase and ALPase signifies damage to tissues [23]. Lowered activity of MDH and LDH indicated less formation of malate and pyruvate in parasite tissue that corresponds to lesser energy yield. The herbal extracts which we have been used increased the phophatase activity (ALPase, ACPase and ATPase) and inhibit the glycolytic enzymes (LDH and MDH) of carbohydrate metabolism. These may prove fatal to parasite by causing damage to tissues and halt energy production of parasite.

Hence it is concluded that these extracts are good candidates for anthelmintic activity.

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