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# *In-Vitro* Effect of Picroliv on Plasmodium Berghei Induced Alterations in the Activity of Adenosine Triphosphatase, Aryl Hyrocarbon Hydroxylase Enzymes and MDA in Liver Explant Culture

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**Abstract**

Activity of adenosine triphosphatasc (ATPase), aryl hydrocarbon hydroxylase (AHH) enzymes and MDA were investigated employing serum-free murine live explant culture from day 0 through 4 following Plasmodium berghei infection (5% parsitaemia). Results demonstrate the maintenance of ATPase activity in the tissue from normal mice, with attenuation noticed in the tissue from infected animalsthroughout the period of culture The enzyme activity also exhibited responsiveness to Picroliv(0.5mg/ml) added to the culture system, The drug metabolizing enzyme (DME) AHH was also expressed in significantly ($p > 0.01$) high amounts in both the type of tissue onday 0 decreasing by 10 fold in 24 ours. MDA level also changes during culture and found a very positive response with picroliv. Subsequently, upto day 4, the activity profile did not show much change. However, Picroliv(0.5mg/ml) responsiveness was found to be better in the tissue culture system in the absence of serum with each explants containing the component cell types of liver makes it a novel approach to evaluate the biochemical basis of enzyme regulation.

**Keywords:** ATPase; AHH; MDA; DME.

## Introduction

Malarial infection remains a problem in tropical and sub-tropical world. The situation has worsened due to the emergence strains resistant to a number of know Antimalarial. Efforts are currently on to develop effective vaccine for the management of thedisease [1].

Derangement of liver metabolism [2]with alterations in blood components [3] is quite elaborately documented in patient infected with acute malarial infection.Picroliv, a known hepatoprotectiv and immunostimulant, isolated from roots and rhizomes of Picrorhizakurroa. Picrolive promotes the secretion of bile flow and can neutralize some of the antigens of hepatitis virus. It enhances the percentage of viable hepatocytes and antagonizes the changes in the enzymes glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) produced by thioacetamide, $CCI_4$ and galactosamine in the isolated hepatocyte suspension [4]. The protective action of picroliv extracts against $CCI_4$ induced hepatotoxicity has also been supported by histopathologicalobservatinos [5]. Immunomodulatory effect of picroliv on the efficacy of paromomycin and miltefosine in combination in experimental visceral leishmaniasis has also found [6].

In order to generate composite information on the basis of enzyme regulation in liver cells in general and the entire organ in particular during malaria, it would be plausible to develop a simple culture system which would allow intercellular component among the component cell types. Since this issue has remained unaddressed so far, we have adopted a simple approach of culturing murine liver explants in serum-free medium for a period of four days. Adenosine triphosphatase (ATPase), a plasma membrane marker enzyme, phase I drug metabolizing enzyme (DME) aryl hydrocarbon hydroxylase (AHH) and MDA level were measured. The studies have been extended to explants obtained for *plasmodium berghei* infected animals.

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## Materials and Methods

### *Chemical*

Adenosine triphosphate(ATP),Glucose-6-phosphate(G6P),Insulin and tissue culture ware were procured from Sigma Chemicals Co, USA, Nicotinamide adenine dinucleotide phosphate (NADP) and Nicotinamide were purchased from SISCO Research Laboratories, Bombay, India, Magnesium chloride, sodium chloride and potassium chloride were obtained from Qualigens Fine Chemicals, Bomboy,M-199 medium and antibiotics were purchased from GIBCO BRI Laboratories (New York, USA). All other chemicals used were of analytical grade.

### *Animals*

Adult male Swiss albino mice weighing about 20-25 g were procured from breeding centre of the institute the animals were kept under standard conditions and had free access to standard pellet diet(lipton Indian Ltd, Bombay) and water *ad libitum.* INSA guidelines were adhered to in the handling and care of the animals. The mice were inoculated i/v with $10^6$sporozoites of *P.berghei* (NK-65 strain) and parasitaemia was monitored daily in Giemsa stained blood smears animals were sacrificed at 5% parasitaemia level in the blood.

### *Culture Medium*

The medium for incubating liver explants was prepared as recommended by Shailubhai*et al* (1990)[7]. with following ingredients; Medium 199,HEPES buffer(10mM,pH-7.4),Penicillin(50IU/ml), Streptomycin(50ug/ml)NaHCO,(175mg) and pH was adjusted to 7.4. Picroliv at a concentration of 0.5mg/ml was added to the medium the medium was sterilizer by filtration through 0.45 micro membrane filters and stored at 4ÚC until use.

### *Culture of Liver Explants*

Animals (normal and *P.berghei* infected) were sacrificed by cervical dislocation. Liver was removed from animals in the laminar flow under sterile conditions. Explants each weighing about 1-2 mg each were cut and approximately 50 explants were incubated in 90 mm sterile petridish, in 20 ml of medium in the and presence of Picrolivdepending upon the protocol. Incubations were conducted at 37ÚC for a period of four days in humidified container. The explants were harvested 0,1,2,3 and 4, washed with chilled 0.15M KCL and stored in liquid nitrogen until use.

### *Homogenization of Liver Explants*

Liver explants were homogenized by sonication in chilled 0.15 MKCl. The homogenates were used for ATPase enzyme assay. One ml of homogenates of each sample was centrifuge at 10,000 xg for 8 min at 4ÚC and the resulting supernatant was used as a source of AHH.

### *Enzyme Assays*

ATPase activity was determined by the of Abdel-Latif et al (1973) [8]. AHH activity was fluorimetrically essentially according to Dehnen*et al [9].*

### *MDA Measurement*

The estimation of lipid peroxides was carried out in post mitochondrial fraction (PMF) according to the method of Utley et al. (1967) [10].

### *Protein Determination*

Protein content was estimated method of Lowry [11] et al (1951) using BSA as a standard.

## Results

### *Adenosinetriphosphatase (ATPase) Activity in Liver Explants from Normal and P. Berghei Infected Mice and its Regulation by Picroliv*

ATPase activity, a plasma membrane enzyme, was monitored in explants cultured from land P. berghei infected mice for a period of four days index of gene expression (Figure 1). Fairly high amount of activity was expressed on day zero which was found well maintained throughout the period of culture in the explants from normal mice. Picroliv at a concentration of 0.5mg/ml was observed to induce the enzyme activity through four days. The induction in enzyme activity was of the order of 8%, and 30% on day 2 and 4 respectively when compared with respective control. On the other hand in the explants obtained from parasitized animals a 30% decrement of the activity was observed on day 1 and 2 of culture after an initial high value on day zero as compared to control. Nevertheless, the extent of activity stabilized on day 3 and 4 of culture the extent of induction of enzyme activity by picroliv in the tissue from the infected animals was similar to that observed in the tissue from

control group on an overall basis. Briefly the enzyme activity was found to be attenuated in the tissue from the infected animal without much change in its inducibility with picroliv in the two cases.

*Arylhydrocarbon Hydroxylase (AHH) Activity in Liver Explants from Normal and P. Berghei Infected Mice and its Regulation by Picroliv*

AHH activity, chosen as a model for the phase I drug metabolizing enzyme apparatus, was evaluated. The tissue was cultured from one through four days in serum free medium harvested on each day and the activity was assayed including day zero. The activity was found to have been expressed in significantly high amount on the day of the initiation of the experiment in the explants from normal animals. Subsequently, a tenfold fall was observed by day 2 following which the enzyme activity remained unchanged upto day 4 of the experiment. In-terms of picrolivresponsiveness of the tissue from normal animals, marked induction of the order of 140 and 190% in AHH activity was noticed on Day 2 and Day 4 respectively. Parasitaemia failed to alter AHH activity in the tissue from the infected animals when compared with corresponding control on Day 0. Subsequent to a similar decrease of 10 fold as in control group on day I, the activity, profile did not reveal much change through day 4 of the experiment in the infected group. Sizableinduction of the enzyme activity upon picroliv treatment was observed (ay 1 = 191%; Day 2= 206%; Day 4 = 83%) In the explants form parasitized animals except on day 3. The overallprofile of AHH activity expressed in the explants was found to be comparable in the tissue cultured from normal and parasitized animals in the absence of Picroliv. In the latter case, inducebility of enzyme activity with Picrolivwas observed to be better (Figure 2).

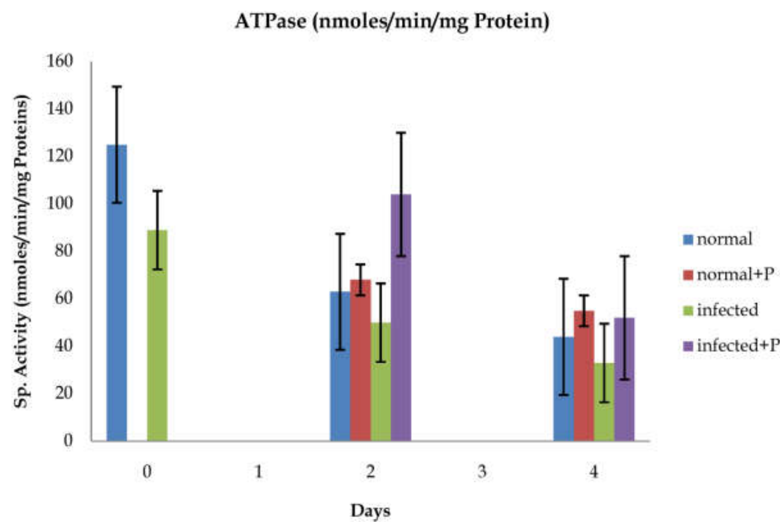


**Fig. 1:** ATPase activity in murine liver explants from normal and 5% parasitaemia induced animal cultured in the absence and presence of Picroliv (0.5 g/ml) upto four days

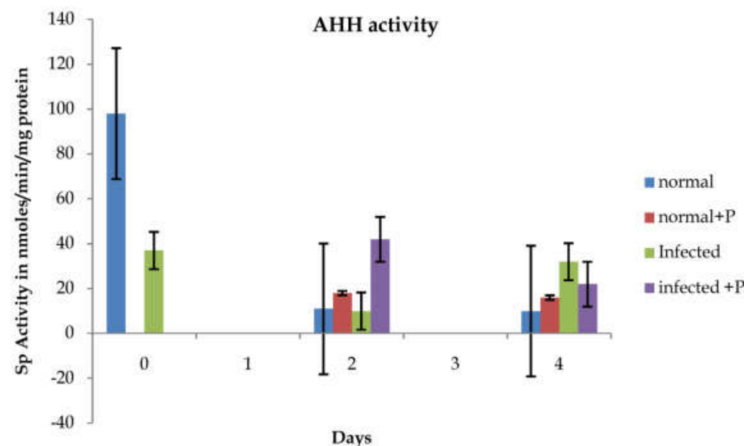


**Fig. 2:** AHH activity in murine liver explants from normal and 5% parasitaemia induced animal cultured in the absence and presence of Picroliv (0.5 g/ml) upto four days

*Malonyldialdehyde Activity in Liver Explants from Normal and P. Berghei infected mice and its regulation by picroliv*

Presence of Picroliv in the culture medium resulted in a decline in the content of lipid peroxidation in the normal control and parasitized cultured liver tissues. However, the extent of response decreased with sequential increase in infection. Decrease in the MDA content was by about 72% in the normal controls at 20% parasitaemia as compared to the respective controls (Figure 3).

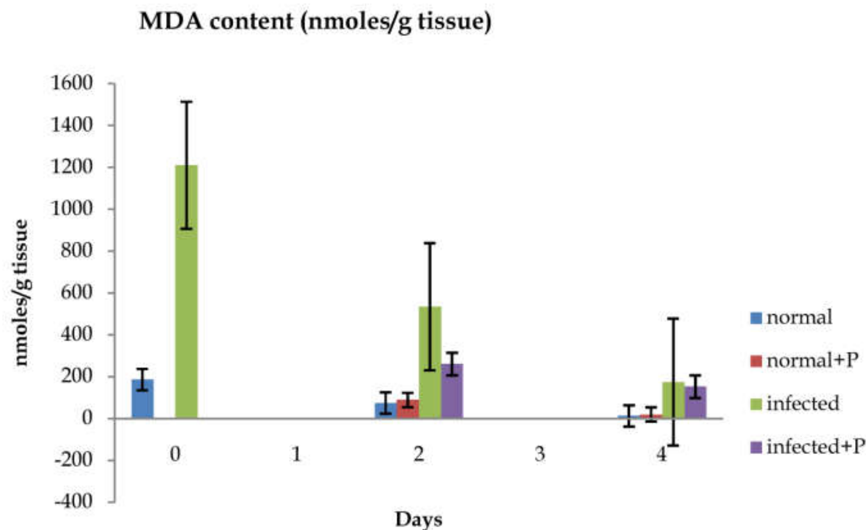


**Fig. 3:** MDA content in murine liver explants from normal and 5% parasitaemia induced animal cultured in the absence and presence of Picroliv (0.5 g/ml) upto four days.

## Discussion

In present study, liver explants from normal and P. berghei infected murine tissue have been cultured in serum free medium for a period of four days. ATPase and AHH enzyme have been used as indicators of changes in the plasma membrane architecture and drug metabolizing enzyme apparatus respectively in the cultured tissue. The culture system accounts from autocrine and paracrine mediated growth factor mechanisms operative amongst the component heterogeneous cells in the explants (Sporn et al, 1985) [12].

The association of hepatomegaly with malaria has been reported by Alvares et al (1984) [2] and kato (1977) [13]. They have evaluated the status of mixed function oxidase (MFO) system in malaria, without investigating the biochemical changes in any marker enzyme of plasma membrane which mediates the parasite entry into the liver cells. The maintenance of a significantly high amount of ATPaseand its inducibility with picroliv in normal murine tissue throughout the culture period in indicative of the viability of our culture system which is pivotal for conduct such studies (Figure 1).

The fact that the enzyme activity and its inducibility with picrolivundergoing down regulation (during parasitaemia as low as 5%) is indicative of the vulnerability of plasma membrane composition to infection. Use of isolated hepatocytes in culture as such of in co-culture with fibrablasts (Michalopoulos et al., 1997) [14] involves problems of proteolytic enzymes usage inflicting membrane damage and the presence of foetal calf serum (FCS) further. Complicating the interpretation of results, such systems have failed to satisfactorily express drug metabolizing. Enzymes etc. (Michalopoulos et al, 1982) [15]. To our knowledge this is the first such report which demonstrates changes in a plasma membrane marker in the tissue cultured without any enzyme treatment in serum – free medium

The changes as a result of low level infection (5%) were limited not only to biochemical changes in the plasma membrane composition but also percolates down to the phase I drug metabolizing enzyme apparatus exemplified by the modulation of activity in the present studies (Figure 2) After aninitial substantial expression of AHH enzyme activity on day 0, the same decreases by about 10 fold from day1 through 4 of the experiment both in normal and experimental groups. The notable part is however, the maintenance of same activity throughout the period of culture. Donato et al have observed a steady decline

in the AHH activity in hepatocytes up to day of culture in the presence of in our case. The expression of enzyme activity remaining from cultured from both the normal and parasitized plus on day 3 in the MFO bio-transformation system ar hepatocyte culture seems to have been overcome by simpleserum free explant culture system. In hepatocytes culture with fibroblast cell line, a better preservation of chrome P450 has been demonstrated as against in pure hepatocyte culture (Paine et al) [16]. Srivastava et al (1991) [17] and Tekwani et al (1988) [18] have reported a decline in the activity of AHH in the liver from parasitized mice although none has addressed tile situation in tissue upon culture as in our case. The explant culture system can therefore be considered akin to the coculture system with the added advantage of the case of set up. It would interesting to evaluate the behavior of phase II components DMEs like Quinone Reductase, Glutathione-S-transferase employing this system. The up-regulation or AHH activity proceed in the tissue from normal and infected animals upon picroliv addition also seems to be interesting. One canspeculatethatup-regulation may be either due to increased transcription and translation of AHH per se or attenuated degradation of enzyme. Further experiments will have to be carried out ascertain the cause (s) of the AHH enzyme up-regulation authors have not come across any literature report thus to demonstrate insulin effect on AHH activity.

Taken together, it can be deduced that even low levels of *P. berghei* infection induces profound changes both at the of plasma membrane and also in the cytosol as evidenced the activities of ATPase and AHH respectively. The chemical changes observed in normal and infected explants be attributed to the interactions occurring in the tissue *per se* the system is essentially serum- free.

The roles played by picroliv (in vitro) in protecting the liver from *P.berghei*induced damage suggests their importance for malaria management [19].

Increased levels of lipid peroxidation and decreased ATPase in liver might be due to impairment of plasma membrane as a consequence *of P.berghei* infection.

In vitro addition of picroliv to the culture medium potentiates the repair mechanisms in the tissue damaged by P. berghei infection in achieving normalcy in items of histo-architecture and biochemical parameters,. The mode of action of hepato-protective and immunostimulant nature of picroliv is still unclear. However, picroliv prevents the generation of lipid peroxides, lipid peroxides resulting in the normalization of biochemical indices.

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