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In Vitro Effect of Chlorquine and Picrolive on DNA Content and Protein-Turnover in Liver and Spleen Cultures After *Plasmodium Berghei* Infection

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Abstract: Background: Malaria is the major cause of disease and death on a global basis. The situation has worsened due to the emergence of parasite strains, resistant to a number of known antimalarials, causing relapse mostly in case of *P vivax*. That's why the great need of combination drug therapy along with known compounds and phytocompound came into the picture for dealing drug resistance by introducing a new therapeutic approach. As liver and spleen are found to play the major roles to overcome the complications during malarial parasitemia, we have focused on the alteration of DNA synthesis and protein content during different levels of parasitemia before and after drug application. The study aims to analyse of the activity of Chloroquine and Picroliv on DNA content and protein turn over in both liver and spleen explants culture after P. berghei infection. Histological preparation of spleen explants for paraplast embedding total DNA content and protein turnover. In liver: In malarial, DNA content is observed to decline rapidly as the parasitemia level increases in liver tissue culture. Decrement of DNA content was found almost 25-52% as the parasitemia level increases 60% up to 0-4 days of culture. After insulin supplementation DNA content was found to increase almost 23% on Day 2 and 33% on day 4. Addition of picroliv enhances DNA content about 32%, 11% on day 2 and 4 respectively. Effect of Chloroquine is also observed with insulin and picroliv administration. Throughout the process, total protein concentration was observed to get decreased in proportion to the rate of infection. At 23% of infection protein values decreased by about 4 of culture respectively over the normal controls. Rate of 3-H-Thymidine incorporation is also observed at different stages of parasitemia. Spleen: In spleen also the same tests were done to observe DNA content at different parasitemia levels, where the reduction of DNA content was observed at 60% infection to be as 11% on day3. After addition of insulin it was increased by 133%; infections. Further increase was also observed about 94%. Total protein concentration was also observed to decrease by 23%, 66% at 20% parasitaemia on day 0 and 3. Total DNA content and total protein content was observed to decrease as the parasitemia level increases in cases of liver and spleen. After addition of insulin, could increase the DNA content slightly, further enhanced by application of picroliv in the media.

Keywords: Malaria, Plasmodium Berghei Parasitemia, DNA Content, Total Protein in Malaria, Chloroquine, Picroliv, 3-H-Thymidine Incorporation

1. Introduction

A winged arthropod, *Anopheles sp.* is one of the 45 varieties of mosquito species worldwide acts as a wreaking havoc for a tropical and subtropical disease, known as Malaria. As early as 1954, WHO reported malaria responsible for deaths of one million infants and young children in rural communities in the tropical belt of Africa yearly. Almost 75 million of positive cases were Hreported in India, 1953 which led to National Malaria Eradication Programme (NMEP) and launched in

1958 for the control measurements [1]. According to WHO, 2019, malaria death was almost 409000, where children under 5 years age are reported as more vulnerable, accounted to be infected is almost 67% which is near about 27400 deaths worldwide [2]. In 2018 almost 228 million positive cases and 4,05,000 were reported globally. Where in India, there are 2 Million cases are registered. Although the graph is reported falling by 219 million worldwide, India could report only 3 million in 2017 [3].

Among the all four species of parasites, *P. falciperum* is the most infectious one developing resistance towards the antimicrobial drugs (Chloroquine, Primoquine etc.), leads to severe life threat [4]. Malarial toxicity is already reported to be found in organs like liver, spleen, kidney, lung and so on, where Hepatomegaly and Splenomegaly is observed to alter the efficiency of liver and spleen in the host body. For that reason the biochemical parameters of liver and spleen are monitored for staging the severity of malaria in the patient [4]. According to scientist Clark and Tomlinson, spleen enlargement is due to the excessive proliferation and fibrosis of lymphoid cells and accumulation of Hb-pigments in the pulp cord. The anti-malarial drugs, like quinine, promaquine, mefloquine, chloroquine etc. mode of action in different stages of infection is still under the question. Picroliv is well known immunoresistant compound which protects liver and spleen cells from malarial inflammation. Its chemical composition consists of irridoid glycosides extracted from the roots of Picrorhiza kurroa. The 60% mixture of Picroside-I and Kutkoside in a ratio of 1:15 and has been known as Picroliv, well known for its hepato-protective roles [5].

In this study we would observe the effect of Chloriquine and Picroliv on Malaria infected hepatic and splenic cells on the basis of DNA content and protein content.

Life cycle of Malaria: Infection initiates with biting of a malaria infected female Anopheles sp. which injects its sporozoites in the host's blood circulation. Sporozoidesrapidly reaches the hepatic system of the affected person and give birth to thousands of Merozoites. Merozoides invades erythrocytes released into the blood stream. The prevention of infection after entering into blood stream is barely possible without any immunization with attenuated sporozoides or by drug treatments [6]. To develop protection against malaria, nearly thousands of repeated exposures of infected mosquitoes are needed. The immune response comes by the presence of lover stage parasites, the trophozoites.

Alteratrions in Liver during Malaria: Liver is known as largest mammalian organ synthesizing major number of drug metabolizing enzymes, receiving the toxic substances which are produced by parasitic infections [7]. Malaria is characterized by hepatomegaly by deposition of heme pigments in the reticuloendothelial system [8] as well as kupffer cells, ultimately causes hypertrophy of liver cells. Hemozoin was found to work as a stimulant to the reticuloendothelial system, causing dysfunction as well as hyper function of the cells.

Alterations in Spleen during Malaria: In malaria, capsules get usually thicker and sub capsular hemorrhages can be visible. On the other hand the inner side of the spleen is observed to become highly congested with higher intra-capsular pressure. The phagocytic macrophages present in splenic cords and sinuses were found to contain malarial pigments where the splenic enlargement is mostly due to the proliferation of lymphoid cells and pigment containing shrunken pulp cords [9].

During the time of *P. berghei* infection, role of spleen is quite important where the splenic lymphocytes respond to antigenic stimuli during parasitic infection. Stimulation of DNA synthesis in explanted spleen cells from an infected rat is an expression of erythropoietic activity, as a result of extensive blood loss during malaria [10]. In the spleen, increase in the activity of Orinithine decarboxylase (ODC) and production of blood cells might be caused by hemolytic anemia. Markedly increased ODC activity might be one of the defense mechanisms in the host to suppress the development of infectious disease by producing lymphocytes in the white pulp and to overcome anemia by producing erythrocytes in the red pulp. Splenic hyperemia is severe and it occurs during the first week of infection.

Role of Insulin in Malaria: A significant role of insulin, normo-glycemia and associated modulations in the activity of Tumor Necrosis Factor (TNF) vis-à-vis malaria has been established. Hypo glycaemia is known to be a serious complication of *P. falciparum* [11]. Administration of glucose as an emergency therapy is unsatisfactory because glucose stimulates insulin release and hypo glycaemia is directly correlated with hyper insulinaemia. The role of insulin in hypo glycaemia has been proven by various workers and has been described Hyperinsulinaemia in hypoglycaemic patients following quinine treatment has also been reported [12].

Activity of Picroliv in Malaria: Picroliv is an indigenously developed, well known hepato-protective and immune-stimulant. It essentially consists of the irridoid glycosides isolated from the roots and rhizomes of *Picrorhiza kurroa*. The rhizomes of *P. Kurroa* contain about 60 percent of a mixture of Picroside-I and Kutkoside in the ratio of 1:15 and has been named Picroliv [13].

Picroliv promotes the secretion of bile flow and can neutralize some of the antigens of hepatitis virus [14]. Picroliv shows dose dependent protective effect on isolated hepatocytes (*ex vivo*) against thio acetamide induced damage in rat. 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 thio acetamide, CCl_4 and galactosamine in the isolated hepatocyte suspension. Picrolive also shows significant protection against hepatic damage due to *Plasmodium berghei* infection in *Mastomys natalensis* [15].

To evaluate the biochemical and histological changes in mice liver and spleen with *P. berghei* infection during malaria and their responsiveness to various ligands, we cultured the normal and *P. berghei* infected liver and spleen explants

under aseptic conditions in *serum-free* medium for 4 and 3 days respectively. The obvious need for undertaking these studies is to throw more light on the biochemical aspects of host liver and spleen metabolism under serum-free culture conditions which has thus far received scant or no attention. Our studies are primarily aimed at elucidating a *serum-free* system for the evaluation of anti-malarial and hepato-protective agents.

2. Material and Methods

2.1. Chemical

Insulin, Glucose-6-phosphate (G6P) 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. India. Nikon Microscope (DFX, Japan) was used for histological studies. All chemicals used were of analytical grade.

2.2. Experimental Animals

Albino Swiss adult male mice, weighing about 20-25 gm. purchased from breeding centre of the institute. The animals were kept under standard laboratory conditions and had free access to standard pellet diet (Lipton India Ltd., Bombay) and water. INSA guidelines were adhered to in the handling and care of the animals.

2.3. Parasite

Plasmodium berghei (NK-15strain) maintained in the Institute was used for inducing the infection in mice for the present study.

2.4. Induction of Experimental Malaria

The blood forms of *P. berghei* were maintained in normal male swiss albino mice by passage of infected blood from the donor infected animals after developing a patent infection. The parasitized blood was drawn from the ocular vein in 3.8% sodium citrate solution. Red blood cells were counted using haemocytometer and a suitable aliquot of inoculums containing $1x10^7$ parasitized erythrocytes per ml blood was administered intrapertioneally into the healthy animals.

2.5. Determination of Parasitaemia

Degree of parastiaemia was evaluated by staining a thin smear of infected blood with Giemsa stain. Parasitized red blood cells were counted under oil immersion lens in 50 different microscopic fields. Percent parasitaemia was calculated from the ratio of parasitized to normal red blood cells.

2.6. Media Preparation

Explants from normal and *P. berghei* infected liver and spleen were cultured in serum free medium 199 (pH 7.4) under aseptic conditions. Antibiotics were supplemented in the medium as follows: Penicilin (100 U/ml) and streptomycin (100 mg/L); HEPES (10 mM) and $NaHCO_3$ (175 mg/l) were added as buffering agents and the pH was adjusted to 7.4 with 1N NaOH. Insulin (1 µg/ml), Picroliv (0.5 mg/ml) and Insulin plus Picroliv (1 µg/ml and 0.5 mg/ml respectively) were added appropriately. The medium was sterilized using 0.45 µM Millipore filtration and stored at 4°C until use [16].

2.7. Tissue Harvest from Animals

Normal adult male swiss mice (weighing about 20-25 g), *P. berghei* infected, both untreated and chloroquine treated were sacrificed by cervical dislocation. Chloroquine (16 mg/kg body weight) was administered orally for 6 days. The sacrificed animals were thoroughly washed and cleaned with soap. The fur was shaven off and the shaved animals were wiped profusely with 70% alcohol. The liver and spleen were excised aseptically in the laminar flow and cut into explants of 1-2 mm^3 (1-2 mg each) size. In each 90 mm petridish, a fixed number (50) of explants were maintained in 20 ml of medium in a humidified CO_2 incubator (90% O_2 and 5% CO_2). The explants from normal and parasitized animals (20%, 40% and 60% parasitamia) were maintained in medium in the absence and presence of Insulin for a period of 4 and 3 days for liver and spleen respectively.

2.8. Maintenance of Tissue

During the course of incubation, medium was replaced with fresh medium every 24 hours in the laminar flow. The cultures were incubated at 37°C in a humidified CO_2 incubator. The explants were harvested from zero through four or three days depending upon the tissue type (spleen), washed with chilled 0.15 M KCI and stored in liquid (-196°C) until use for enzyme assays.

2.9. Biochemical Studies

2.9.1. Homogenization of Tissue Explants

Explants from liver and spleen were sonicated in chilled 0.15M KCI and stored on ice. One ml of homogenate was centrifuged at 90000xg for 10 minutes to obtain the post mitochondrial fraction [17].

2.9.2. DNA Estimation

To one ml of homogenate of liver and spleen, 2 ml chilled 20% TCA was added and kept at 4°C for 4 hours. Subsequently, the contents were centrifuged at 4000 rpm and pellet was resuspended in 1 M KOH. After incubating for one hour at 37°C, 3 ml chilled 20% TCA was added. The pellet was crushed and suspended in 0.5M PCA and incubated at 90°C for 10 imutes. The supernatant was collected after centriguation and used for quantifying the DNA content according to Burton (1956) using calf thymus DNA as

standard. The absorbance was read at 600 nm in a spectrophotometer.

2.9.3. C. Total Protein Determination

Total protein content was evaluated in homogenate and PMF wherever required according to the method described by Lowry *et al.* (1951) using bovine serum albumin as standard. Equal volume of chilled 10% TCA was added to the homogenate/PMF and kept at 4°C for 1 hour. Subsequently, the contents were centrifuged at 4000 rpm for 20 minutes at 4°C. The pellet was dissolved in 0.1N NaOH and a suitable aliquots were taken and alkaline copper reagent was added. The reactants were incubated for 10 minutes at room temperature and the absorbance was read at 700 nm after addition of the Folin-ciocalteu reagent.

2.10. Statistical Evaluation of Data

Statistical evaluation of the data was carried out according to Bancroft (1963) using Student's 't' test. 'P' values for statistical significance were calculated by comparing the data of infected mice phase wise against the levels of control animals. 'P' values less than 0.02 are regarded as statistically significant variations against normal values. Values greater than 0.02 regarded as statistically insignificant.

3. Results

3.1. In Liver

3.1.1. Regulation of DNA Content on P. berghei Infection

During malarial infection, there was a steep decline in DNA content with increasing parasitaemia as evaluated in the cultured liver tissue (Figure 1). Decrement in DNA content was 8.6%, 22% and 25% at 20% parasitaemia (NH^{C} Vs $NH^{20\%}$); 19%, 42%, 46% at 40% parasotaemia (NH^{C} Vs $NH^{40\%}$) and 25%, 49%, 52% at 60% parasitaemia (NH^{C} Vs $NH^{60\%}$) on day 0, 2 and 4 of culture respectively (Table 1).

Insulin supplementation to the incubation medium recorded a promotion by about 23% on day 2 (NH_2^{C} Vs I_2^{C}) and 33% on day 4 (NH_4^{C} Vs I_4^{C}) in the explants derived from normal control animals. DNA content increased by about 20% ($NH_2^{20\%}$ Vs $I_2^{20\%}$), 22% (NH_4^{20} Vs $I_4^{20\%}$) at 20% parasitaemia; 4% and 3% at 40% parasitaemia and 19% ($NH_2^{60\%}$ Vs $I_2^{60\%}$), 51% ($NH_4^{60\%}$ Vs $I_4^{60\%}$) at 60% parasitaemia on day 2 and 4 of incubation respectively (Figure 1).

Addition of picroliv to the culture medium demonstrated a significant effect on the DNA content of the cultured tissue in the normal control as well as in infected group from day 2 to 4. In the normal control group increase in the DNA level was of about 32%, 11% on day 2 and 4 respectively (NH^{C} Vs P^{C}). Maximum increment recorded at 20% parasitaemia was 52% and 79% on day 2 and 4 of incubation ($NH^{20\%}$ Vs $P^{20\%}$). With the increase in the extent of infection, picrolive was found to be effective in stimulating the DNA turnover. Under similar incubation condition, DNA turnover was found to increase by 29%, 64% and 40% parasitaemia ($NH^{40\%}$ Vs $P^{40\%}$) and 20%, 46% at 60% parasitaemia ($NH^{60\%}$ Vs $P^{60\%}$) on day 2 and 4 of culture respectively (Figure 4).

Introduction of IP in the medium failed to upregulate the DNA content in normal control group upto day 2. Incubation of the tissue from the infected animals under similar incubation conditions on day 2 increased the DNA content by 15% ($NH_2^{20\%}$ Vs $IP_2^{20\%}$), 17% ($NH_2^{40\%}$ Vs $IP_2^{40\%}$) and 46% ($NH_2^{60\%}$ Vs $IP_2^{60\%}$) at 20%, 40% and 60% parasitaemia levels respectively. On day 4, the effect of IP was more pronounced in terms of DNA turnover which increases by 29% for control (NH_4^{C} Vs IP_4^{C}), 43% for 20% ($NH_4^{20\%}$ Vs $IP_4^{20\%}$), 46% for 40% ($NH_4^{40\%}$ Vs $IP_4^{40\%}$) and 56% for 60% ($NH_4^{60\%}$ Vs $IP_4^{60\%}$) erthrocytic infection (Figure 6).

3.1.2. Regulation of DNA Content by Chloroquine (CQ) and Picroliv on P. berghei Infection

Chloroquine is the most commonly employed drug for the management of malaria whose mechanism of action remains elusive to date. Elucidating the mode of action of CQ, its role in regulating the turnover of DNA was also investigated (Table 1).

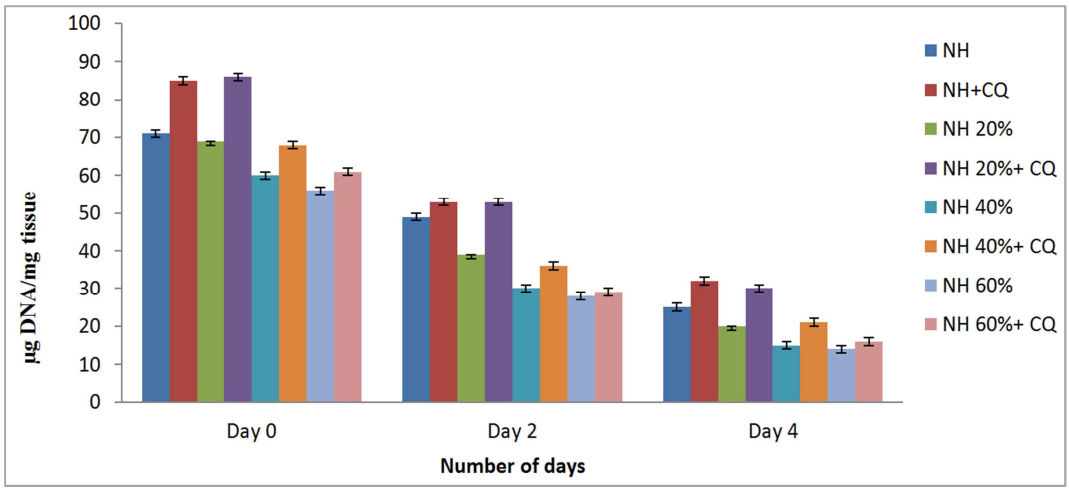

Figure 1. *Total DNA content in P. berghei infected murine liver explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

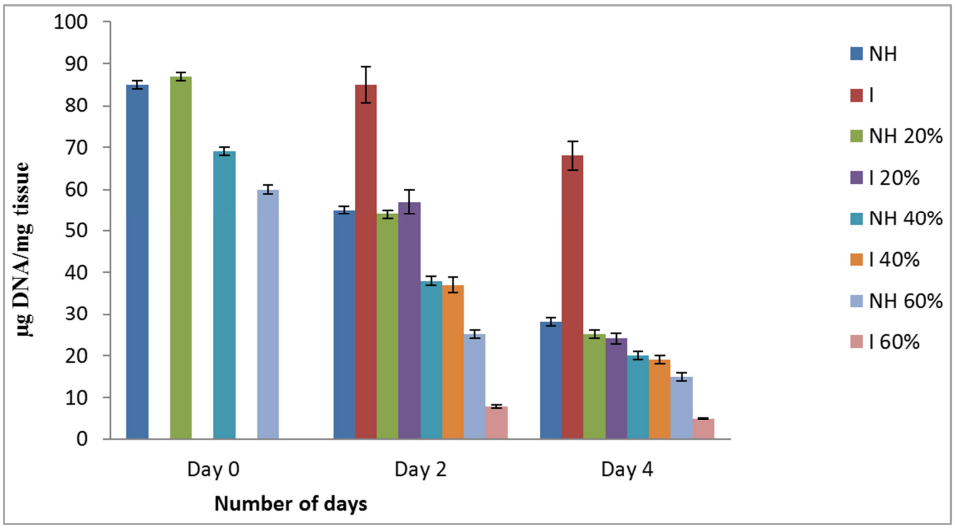

Figure 2. *In vitro effect of Insulin (INS, 1µg/ml) on total DNA content in P. berghei infected, without Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia levels.*

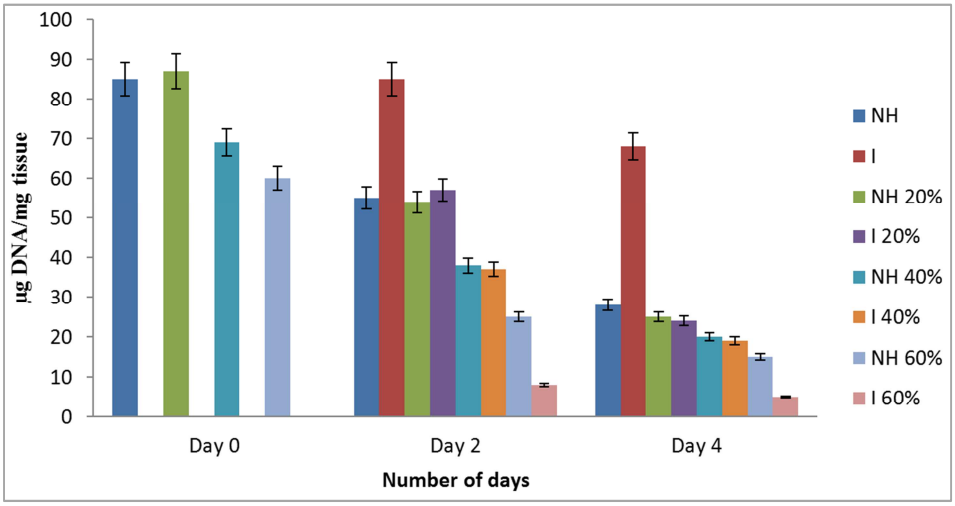

Figure 3. *In vitro effect of Insulin (INS, 1µg/ml) on total DNA content in P. berghei infected, without & with Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia levels.*

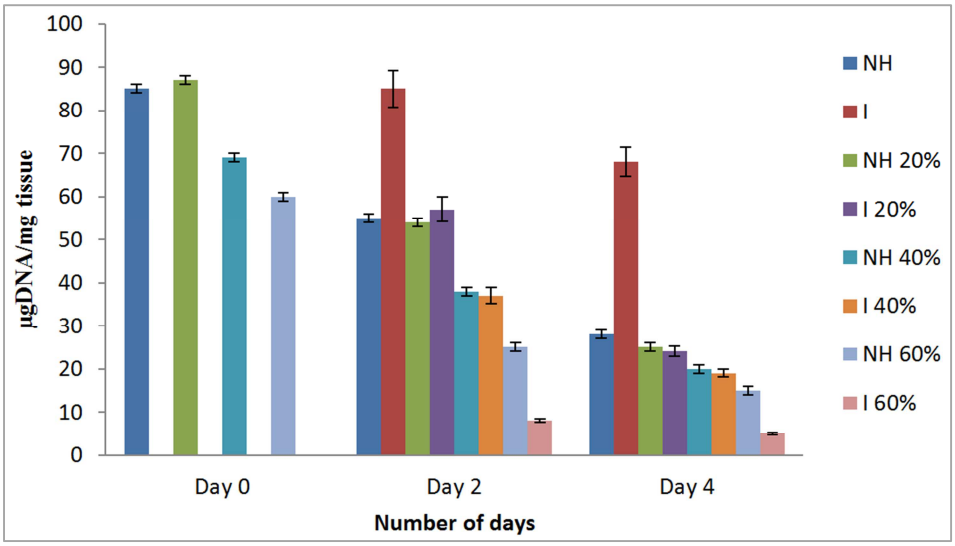

Figure 4. *In vitro effect of Picroliv (PIC, 0.5mg/ml) on total DNA content in P. berghei infected, without Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia level.*

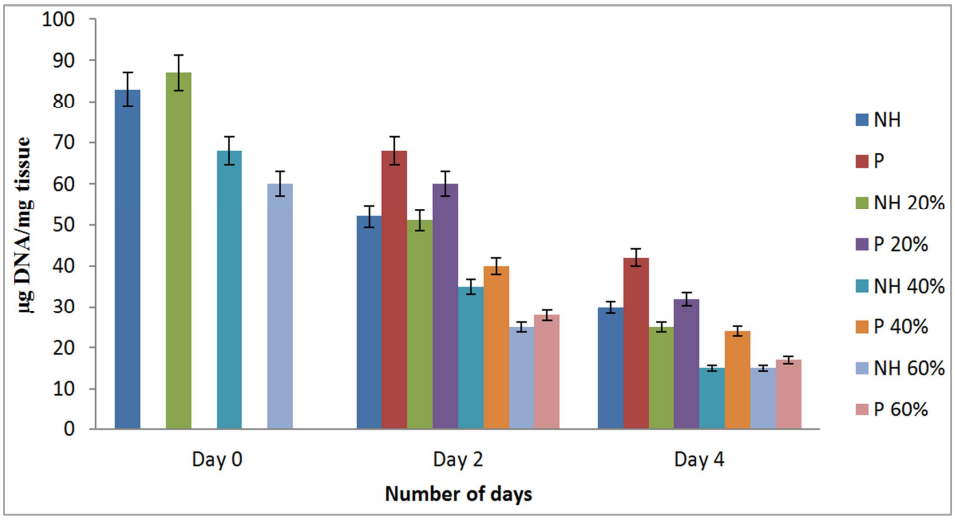

Figure 5. *In vitro effect of Picroliv (PIC, 0.5mg/ml) on total DNA content in P. berghei infected, without & with Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia level.*

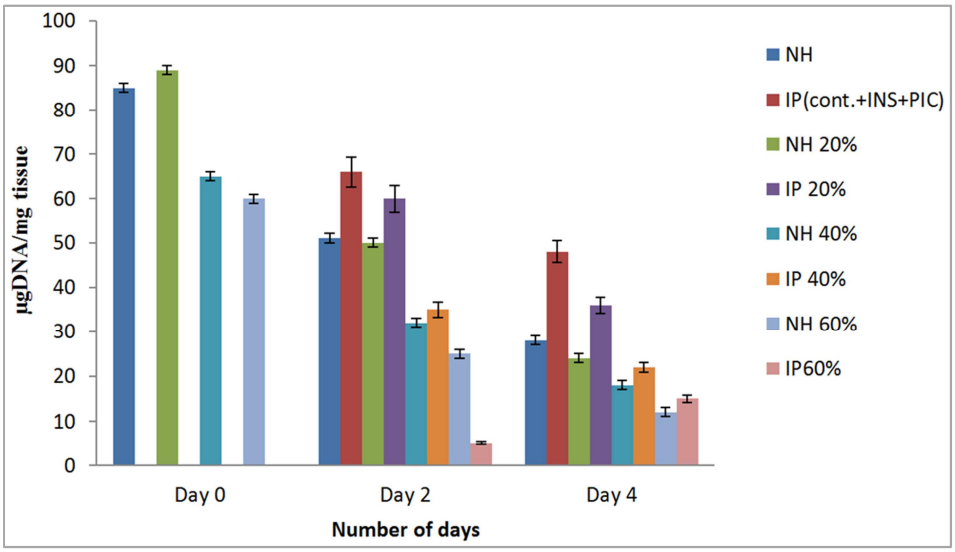

Figure 6. *In vitro effect of Insulin (INS, 1µg/ml) + Picroliv (PIC, 0.5mg/ml) on total DNA content in P. berghei infected, without Chloroquine (CQ) treatment, murine Liver explants cultured in serum-free medium at different parasitemia levels.*

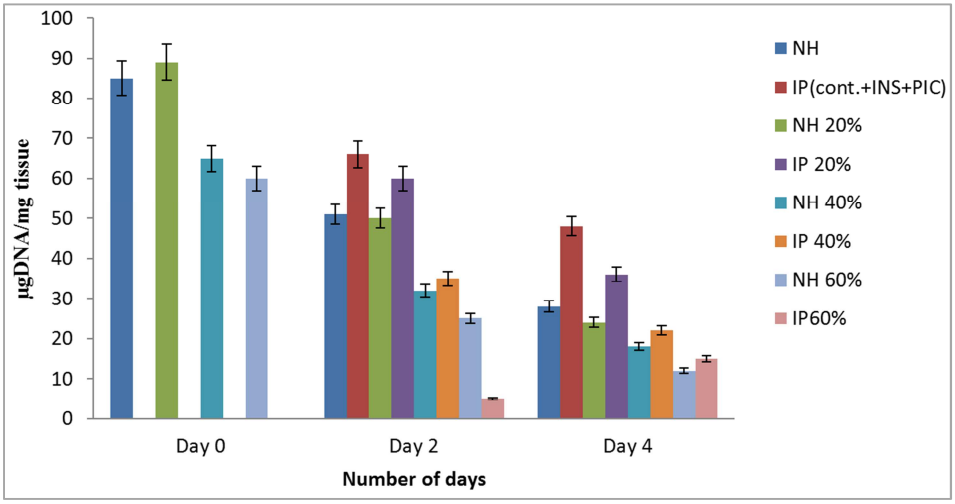

Figure 7. *In vitro effect of Insulin (INS, 1µg/ml) + Picroliv (PIC, 0.5mg/ml) on total DNA content in P. berghei infected, without & with Chloroquine (CQ) treatment, murine Liver explants cultured in serum-free medium at different parasitemia levels.*

Table 1. *DNA content (µg/mg tissue) in liver explants cultured under serum-free condition.*

Experiment	Control	Control+ CQ	20% parasitemia	20% parasitemi + CQ	40% parasitemia	40% parasitemia + CQ	60% parasitemia	60% parasitemia + CQ
NH_0	72.1±3.3	85.5±4.1	68.8±5.2 (8.6)	86.9±2.9 (32)	59.5±2.5 (19)	67.3±3.3 (16)	54.7±4.3 (25)	29.6±2.1 (9)
NH_2	48.5±2.2	52.6±2.6	37.9±1.5 (22)	52.4±3.1 (39)	28.3±6.1 (42)	34.04±2.2 (20)	25.02±3.2 (49)	25.4±1.5 (17)
NH_4	22.32±1.2	30..2±2.8	17.40±3.3 (25)	25.5±1.2 (47)	128±1.2 (46)	18.3±1.1 (43)	10.9±3.9 (52)	13.43±2.3 (23)
I_2	59.4±5.0	85.0±4.1 (62)	45.5±4.0 (20)	55.31±2.2 (5.8)	27.2±1.4 (4)	33.6±1.6 (-1.2)	20.29±3.1 (19)	7.09±1.1 (-32)
I_4	29.6±2.5 (33)	65.2±2.9 (115)	21.31±2.0 (22)	25.0±2.8 -	12.4±1.9 (3)	17.5±1.5 (-2.8)	5.26±1.3 (51)	5.03±1.2 (-52)
P_2	64.2±2.6* (32)	70.5±3.9 (34)	57.22±2.8* (52)	61.3±1.6 (-17)	36.3±2.2 (29)	39.7±2.2 (-16.8)	29.9±1.9 (20)	28.1±2.8 (10.6)
P_4	24.7±3.4 (11)	43.7±5.8 (45)	31.13±2.1 (79)	34.7±1.3 (-36)	21.04±4.1 (64)	22.7±2.1 (-24)	16.1±1.2 (46)	15.6±1.2 (16.2)
IP_2	51.1±1.8 (5.2)	67.9±3.6 (29)	43.4±5.1 (15)	62.4±3.3 (-198)	32.9±2.2 (17)	36.3±3.6 (5.9)	36.8±3.3 (46)	6.18±1.0 (-75)
IP_4	28.8±1.9 (29)	46.7±2.2 (55)	24.8±3.1 (43)	37.3±3.0 (46)	18.9±1.8 (46)	23.4±3.1 (28)	17.1±2.0 (56)	15.1±1.2 (12)

In the tissue incubated from normal controls, CQ treatment enhanced the DNA content insignificantly. However, in the CQ treated parasitized groups the increase was found to be significant as compared to untreated parasitized control. It is interesting to note that the increase in DNA content in the CQ treated parasitized tissue was not commensurate with the increase in parasitaemia i.e. 20.>40.>60. At 20% parasitaemia, enhancement in the DNA content was 32%, 39%, 47% on day 0, 2 and 4 respectively (NH^C Vs $NH^{20\%}$). At 40% infection DNA level increased by 16%, 20% and 43% (NH^C Vs $NH^{40\%}$) and 60% infection 9%, 17% and 23% (NH^C $NH^{60\%}$) on the respective days of incubation i.e. 0, 2 and 4. This, therefore, suggests that the intensity of the lesion following parasitaemia determines the extent of increase in DNA content which follows a reverse pattern i.e. less increase in DNA with increasing parasitaemia (Figure 1).

After the incubation of explants from normal animals in insulin containing medium, DNA content increased by 62% an 115% in the normal control (NH^C Vs I^C) on day 2 and 4, whereas in the tissue from infected group insulin failed to enhance the DNA content. Abatement of increase of total DNA content was noticed with the progression in parasitaemia. No significant alteration of DNA content was found on day 2, 4 of incubation at 20% and 40% parasitaemia. Only at 60% parasitaemia about 32% and 52% ($NH^{60\%}$ Vs $I^{60\%}$) decrease, a significant amount, in the DNA content has been observed on the respective days of culture period (Figure 3).

After the incubation of explants in picroliv containing medium, a protective effect was observed. In the normal group, response of picroliv was more prominent which was found to decrease with the increase in infection. In the normal liver explants, increase in DNA level was 34% (NH_2^C Vs P_2^C) and 45% (NH_4^C Vs P_4^C) whereas at 20%, 40% and 60% infection no significant increase in DNA content was found on either days of incubation (Figure 5).

IP treatment increased the DNA content in normal control group significantly over the untreated control (about 29% and 55%), whereas in the infected group significant increment of DNA content was noted only on day 4 of incubation at 20-60% parasitaemia as compared to the respective control. Increase in DNA content was of the order of 46% at 20% ($NH_4^{20\%}$ Vs $IP_4^{20\%}$), 28% at 40% ($NH_4^{40\%}$ Vs $IP_4^{40\%}$) and 12% at 60% parasitaemia levels ($NH_4^{60\%}$ Vs $IP_4^{60\%}$) on day 4 of incubation of the tissue respectively (Figure 7).

3.1.3. Changes in Protein Turnover During P. berghei Infection

During the entire culture period the level of tissue protein displays a downward trend and the decline is proportional to the extent of infection. At 20% infection protein values decreased by about 27%, 15% and 23% (NH^C Vs $NH^{20\%}$) on days 0, 2 and 4 of culture respectively over the normal controls. When the parasitaemia rises up to 40%, lowering of the total protein was 42% (NH_0^C Vs $NH_0^{40\%}$), 28% (NH_2^C Vs $NH_2^{40\%}$), 27% (NH_4^C VS $NH_4^{40\%}$); at 60% infection the decrease was 45%, 44%, 28% on day 0, 2 and 4 of incubation (NH^C Vs $NH^{60\%}$) (Figure 8 and Table 2).

Table 2. *Total protein content in P. berghei infected murine liver explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

Experiment	Control	Control+ CQ	20% parasitemia	20% parasitemia+ CQ	40% parasitemia	40% parasitemia +CQ	60% parasitemia	60% parasitemia + CQ
NH_0	6.40±1.3	7.77±0.5	4.69±.004*** (27)	7.23±1.2*** (55)	3.73±0.01*** (42)	5.98±0.19*** (50.5)	3.55±0.5*** (45)	5.2±1.7 (44)
NH_2	3.90±0.05	4.71±0.51	3.33±.006*** (15)	5.2±.21*** (55)	2.82±0.06*** (28)	4.53±0.20* (58.2)	2.2±0.12*** (43.6)	3.4±0.1** (55)
NH_4	2.5±0.18	2.63±0.07	1.93±.003*** (23)	2.99±.45*** (54.8)	1.83±.008*** (27)	2.84±0.62 (56)	1.8±.009*** (28)	2.7±0.18** (53)

Experiment	Control	Control+ CQ	20% parasitemia	20% parasitemia+ CQ	40% parasitemia	40% parasitemia +CQ	60% parasitemia	60% parasitemia + CQ
I_2	3.1±0.005 -	4.85±0.07 (4)	3.03±0.005 (9)	3.4±0.23 (-35)	2.8±0.009 -	4.1±0.5 (-9.5)	3.4±0.10** (54)	3.57±0.68 (5)
I_4	1.9±0.22 -	3.46±0.009*** (32)	0.75±0.002 -	2.3±0.01 (-23)	2.17±0.03*** (18)	2.4±0.06 (-15)	3.2±0.08 (77)	1.97±0.02 (-27)
P_2	5.9±0.015*** (51)	4.26±0.01 (-9.4)	4.2±.001*** (26)	2.9±0.2 (-44)	4.1±0.2*** (45)	3.4±0.08 (-25)	4.11±0.52*** (87)	3.5±0.55 (3)
P_4	6.3±0.023*** (152)	2.6±0.008 -	2.8±0.01*** (46)	2.4±0.1 (-19)	2.8±0.42 (55)	1.02±0.04 (-64)	3.44±0.39*** (93)	3.1±0.08 (14)
IP_2	4.8±0.041*** (23)	5.8±0.05 (23)	4.3±.008*** (30)	4.1±.019 (-21)	3.9±0.68 (38)	4.8±0.03 (6)	3.5±0.36*** (59)	3.5±0.06 (3)
IP_4	5.5±0.09*** (120)	3.43±0.088*** (30)	2.9±.004*** (50)	3.5±0.2 (15)	3.1±0.19 (69)	2.7±0.24 (5.3)	3.1±0.32* (74)	2.4±0.05 (-11)

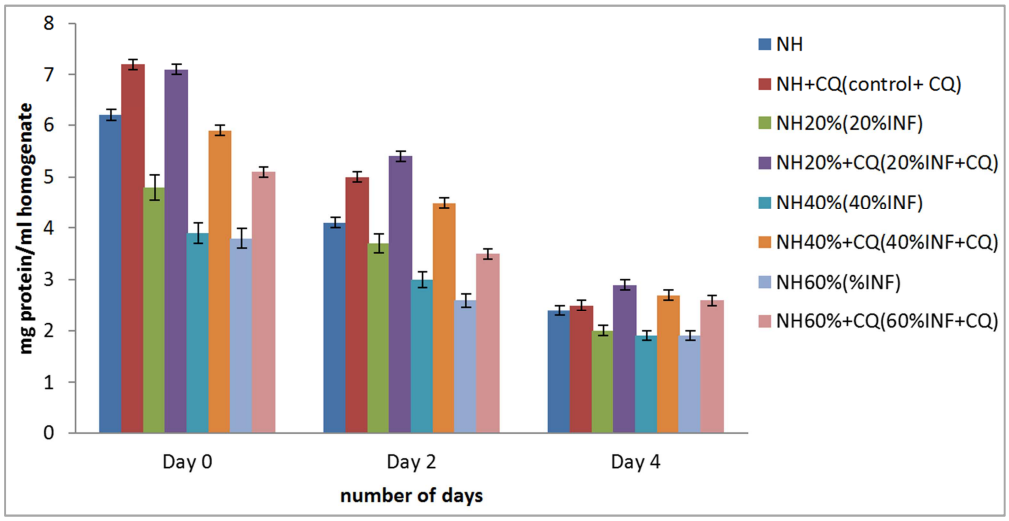

Figure 8. *Total protein content in P. berghei infected murine liver explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

Incubation of the explants in insulin containing medium failed to reverse the decrease in protein content in the normal control group as well as in the 20% parasitized group. The effect of insulin became noticeable with the rise in parasitaemia i.e. at 40% and 60% infection. On the fourth day of incubation, insulin increased the protein content by 18% at 40% malarial infection over the respective control group ($NH_4^{40\%}$ Vs $I_4^{40\%}$). At 60% parasitaemia protein content increased by about 54% ($NH_2^{60\%}$ Vs $I_2^{60\%}$) and 77% ($NH_4^{60\%}$ Vs $I_4^{60\%}$) on the day 2 and 4 of culture due to insulin treatment (Figure 9).

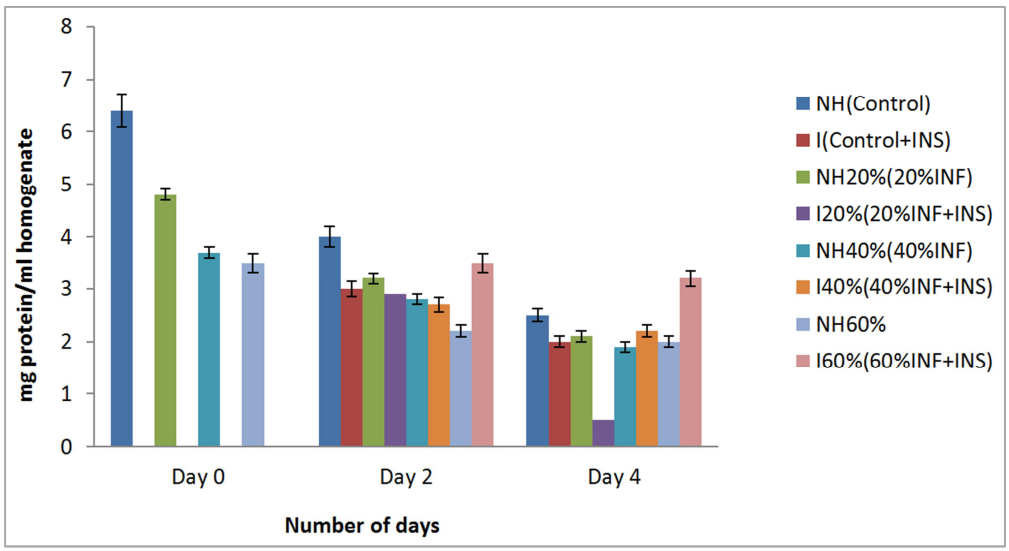

Figure 9. *In vitro effect of Insulin (INS, 1µg/ml) on total protein content in P. berghei infected, without Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitaemia levels.*

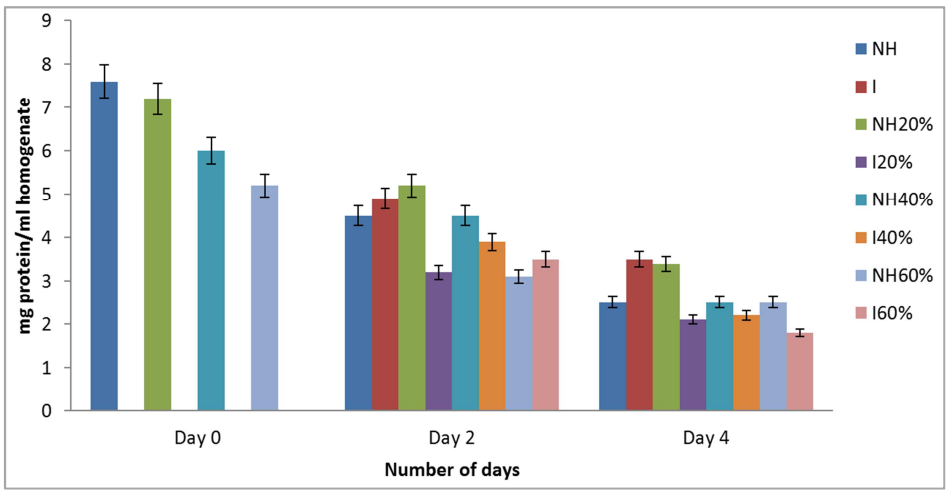

Figure 10. *In vitro effect of Insulin (INS, 1µg/ml) on total protein content in P. berghei infected, without and with Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitaemia levels.*

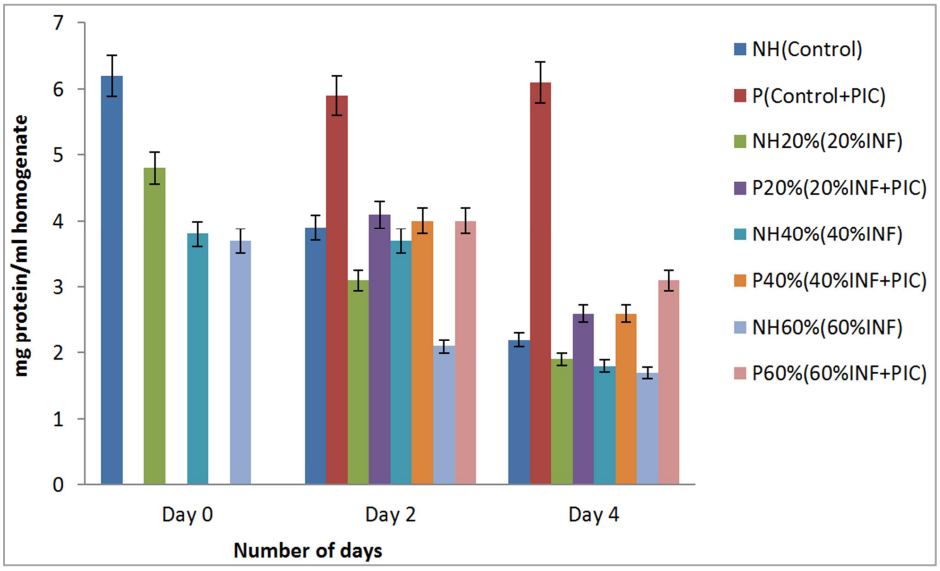

Figure 11. *In vitro effect of Picroliv (PIC, 0.5mg/ml) on total protein content in P. berghei infected, without Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia levels.*

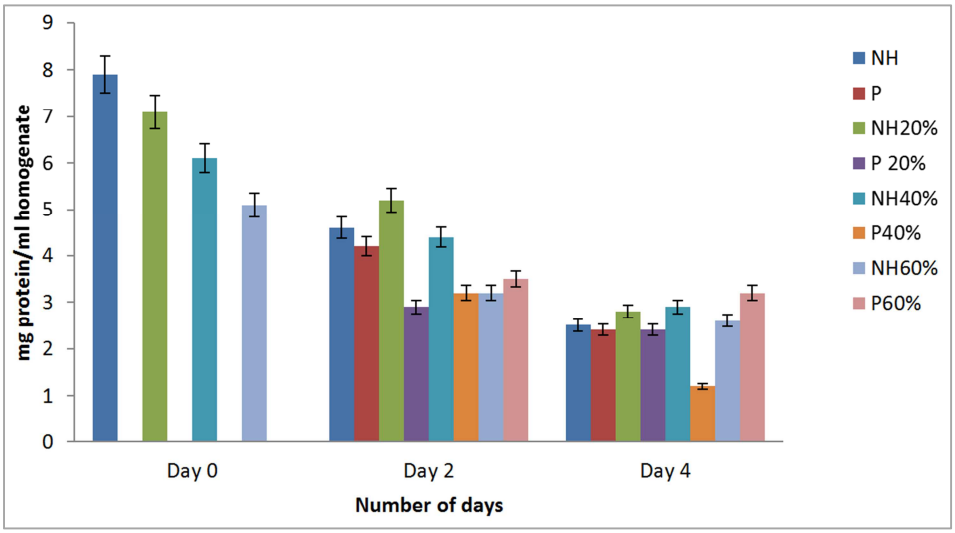

Figure 12. *In vitro effect of Picroliv (PIC, 0.5mg/ml) on total protein content in P. berghei infected, without and with Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia levels.*

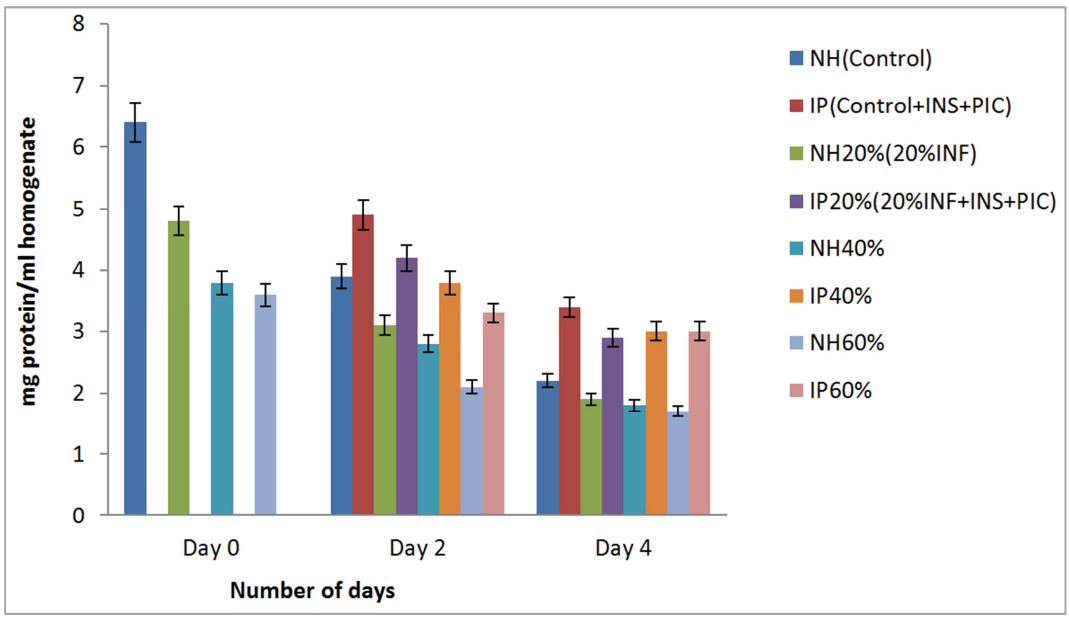

Figure 13. *In vitro effect of Insulin (INS, 1µg/ml) + Picroliv (PIC, 0.5mg/ml) on total protein content in P. berghei infected, without Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia level.*

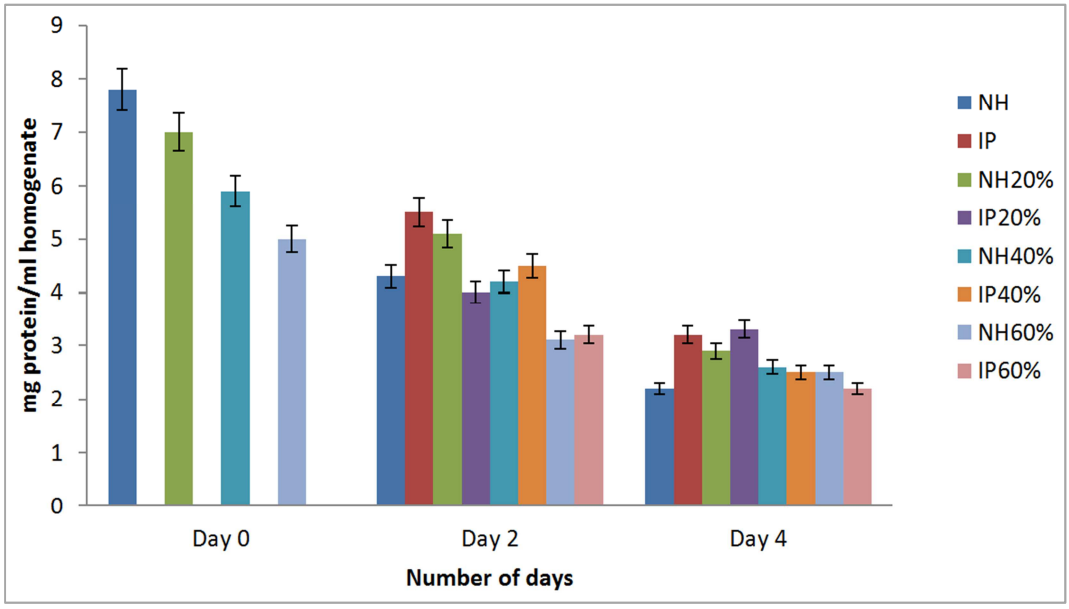

Figure 14. *In vitro effect of Insulin (INS, 1µg/ml) + Picroliv (PIC, 0.5mg/ml) on total protein content in P. berghei infected, without & with Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia level.*

Supplementation of picroliv to the liver explants in culture demonstrated the maintenance of protein throughout the culture period. In the explants from normal control liver, the protein content increased by 51% and 152% on day 2 and 4 (NH^{C} Vs P^{C}). At 20% parasitaemea, the increase in protein content over the respective control was 26%, 46% ($NH^{20\%}$ Vs $P^{20\%}$); 45%, 55% at 40% parasitaemea ($NH^{40\%}$ Vs $P^{40\%}$) and 87%, 93% at 60% parasitaemea ($NH^{60\%}$ Vs $P^{60\%}$) on day 2 and 4 respectively (Figure 11).

In the normal control group, presence of insulin plus picroliv (IP) stimulated the protein turnover by 23% (NH_2^{C} Vs IP_2^{C}) and 120% (NH_4^{C} Vs IP_4^{C}) on day 2 and 4 of incubation as compared to no hormone controls. In the parasitized group under similar incubation conditions, a generalized increase of 30%, 50% at 20% infection ($NH^{20\%}$ Vs $IP^{20\%}$); 38%, 69% at 40% infection ($NH^{40\%}$ Vs $IP^{40\%}$); 59%, 74% at 60% infection ($NH^{60\%}$ Vs $IP^{60\%}$) was noticed as compared to respective controls (Figure 13).

3.1.4. Protein Turnover After Chloriquine (CQ) and Picroliv Treatment on P. berghei Infection

After CQ treatment, a significant increase in protein content was noticed in the explants cultured from parasitized group versus normal controls (Table 2). In the CQ treated parasitized animals with 20% infection the protein content in the cultured liver explants increased by approximately 55%

during four days of incubation (NH^C Vs $NH^{20\%}$). At 40% infection under similar conditions the increment was of about 50-60% during the tenure of the experiment (NH^C Vs $NH^{40\%}$). Similarly at 60% parasitaemia, protein content increased by 44%, 55%, 53% on day 0, 2 and 4 of culture respectively (NH^C Vs $NH^{60\%}$) (Figure 8).

The role of insulin in augmenting the protein content in the tissue cultured from normal and parasitized animals was investigated. In the normal controls, insulin stimulated the protein content by 4%, 32% on day 2 and 4 as compared with NH_2 and NH_4. Conversely, the presence of insulin in the culture system containing explants from CQ treated animals failed to enhance the protein content (Figure 10).

Picroliv supplementation to the culture medium proved ineffective in significantly altering the protein level at any of the parasitaemia levels tested (20%, 40% and 60%) (Figure 12).

Presence of insulin plus Picroliv (IP) in the incubation medium under similar conditions increased the protein content by 15% ($NH_4^{20\%}$ Vs $IP_4^{20\%}$), 6% ($NH_2^{40\%}$ Vs $IP_2^{40\%}$) at 20% and 40% parasitaemia levels on day 2 and 4 respectively only. No effect was noticed in the 60% parasitaemia group (Figure 14).

3.1.5. ^{3}H-Thymidine Incorporation in Liver Explants During P. berghei Infection

Rate of ^{3}H-Thymidine incorporation in liver explants decreased during malarial infection (Table 3). Decrement in the incorporation of ^{3}H-Thymidine in the explants was 43%, 36%, 23% at 20% parasitaemia (NH^C Vs $NH^{20\%}$); 45%, 38%, 24% at 40% parasitaemia (NH^C Vs $NH^{40\%}$) and 50%, 41%, 41% in 60% parasitaemia (NH^C Vs $NH^{60\%}$) on day 0, 2 and 4 respectively when compared to the normal control group (Figure 15).

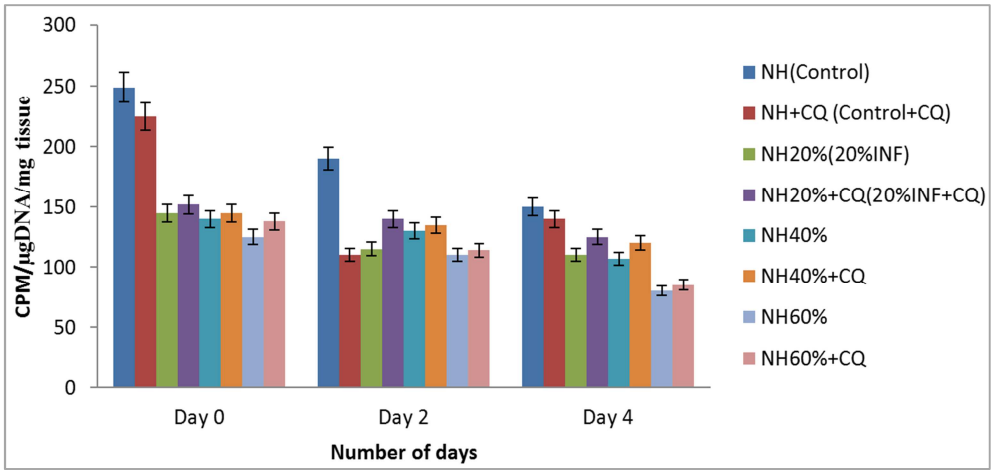

Figure 15. *3H-Thymidine incorporation in P. berghei infected murine liver explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

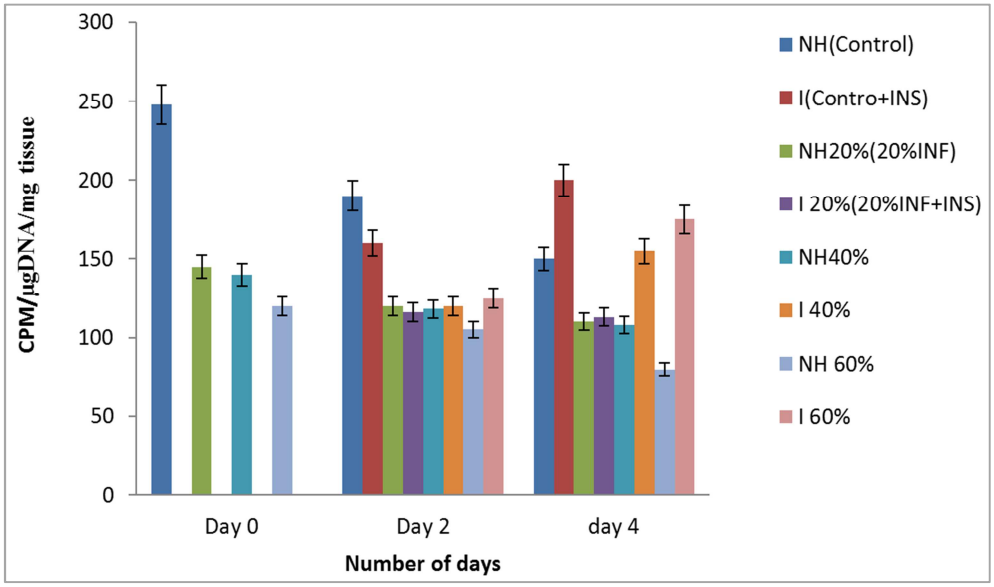

Figure 16. *In vitro effect of Insulin (INS, 1µg/ml) on 3H-thymidine incorporation in P. berghei infected, without Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia levels.*

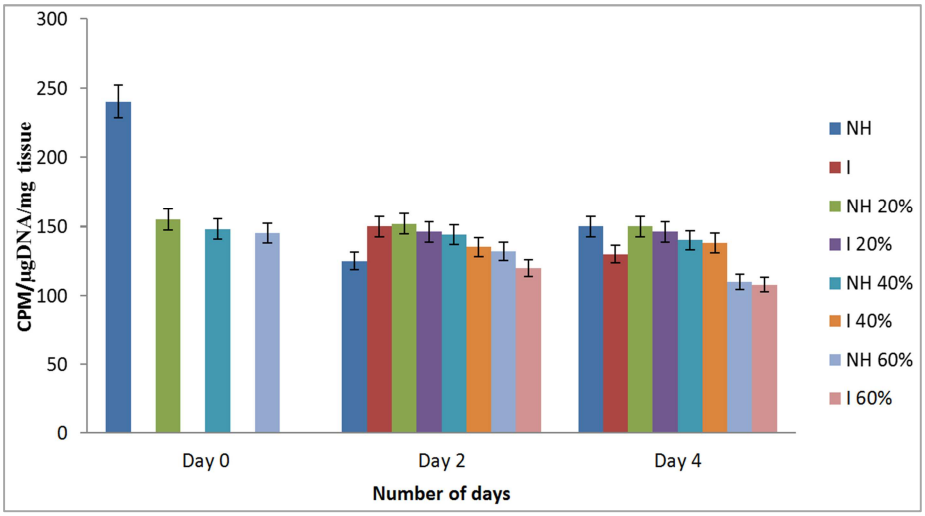

Figure 17. *In vitro effect of Insulin (INS, 1µg/ml) on 3H-thymidine incorporation in P. berghei infected, without & with Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia levels.*

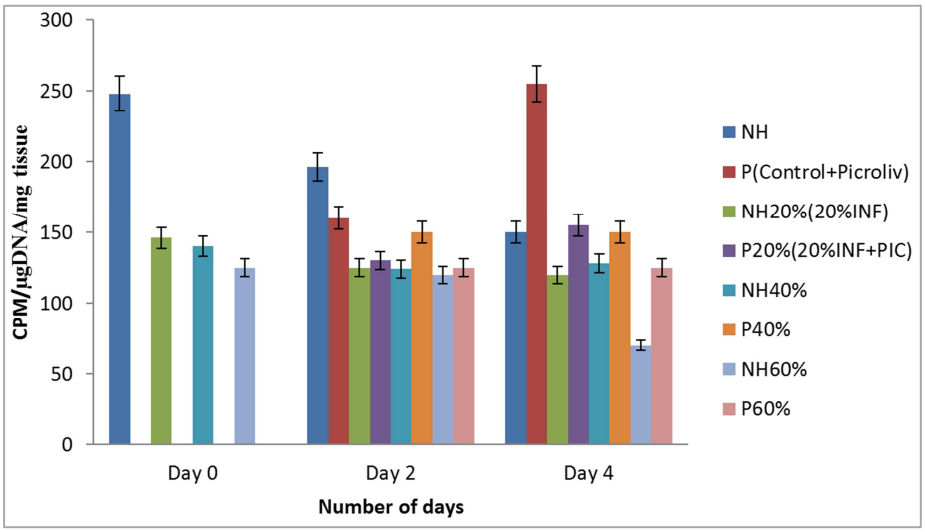

Figure 18. *In vitro effect of Picroliv (PIC, 0.5mg/ml) on 3H-thymidine incorporation in P. berghei infected, without Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia levels.*

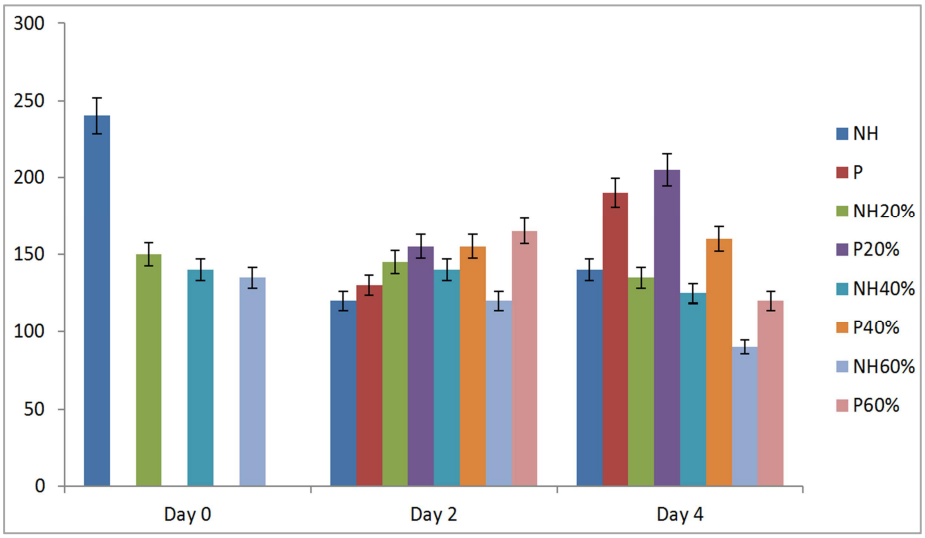

Figure 19. *In vitro effect of Picroliv (PIC, 0.5mg/ml) on 3H-thymidine incorporation in P. berghei infected, without & with Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia levels.*

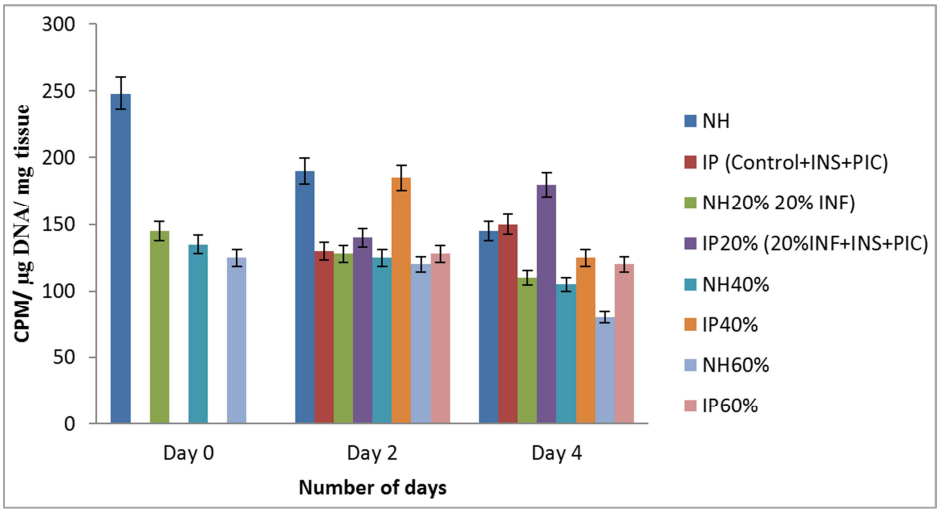

Figure 20. *In vitro effect of Insulin (INS, 1µg/ml)+ Picroliv (PIC, 0.5mg/ml) on 3H-thymidine incoration in P. berghei infected, without Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia levels.*

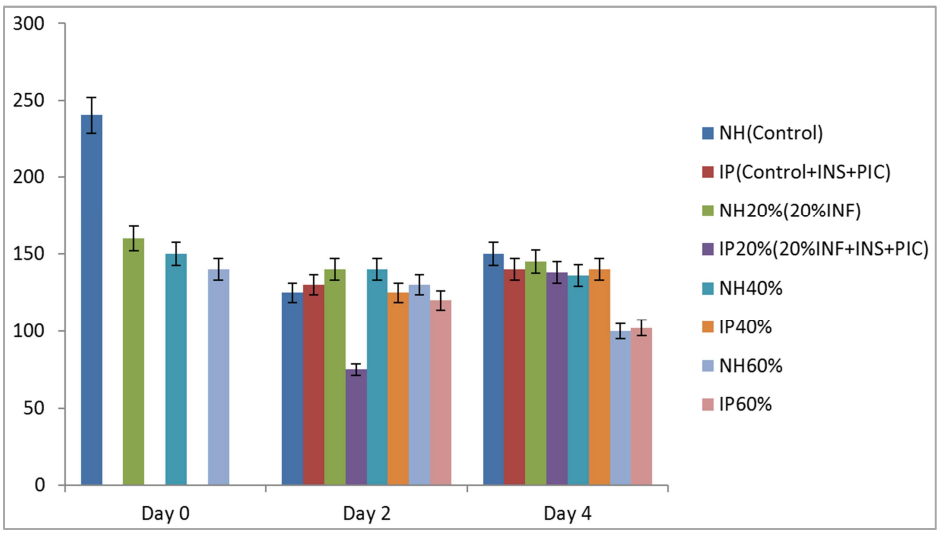

Figure 21. *In vitro effect of Insulin (INS, 1µg/ml)+ Picroliv (PIC, 0.5mg/ml) on 3H-thymidine incoration in P. berghei infected, without & with Chloroquine (CQ) treatment, murine liver explants cultured in serum-free medium at different parasitemia levels.*

Table 3. *3H-Thymidine incorporation in P. berghei infected murine liver explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

Experiment	Control	Control+ CQ	20% parasitemia	20% parasitemia+ CQ	40% parasitemia	40% parasitemia+ CQ	60% parasitemia	60% parasitemia+ CQ
NH_0	247.0±8.2	229.4±7.6	141.9±4.5*** (43)	152.2±5.2 (8)	137.0±2.5*** (45)	144.0±3.1 (5)	124.0±6.2*** (50)	132.9±6.4 (7)
NH_2	189.7±9.6	112.4±9.1	122.1±10.0* (36)	135.5±4.1 (11)	119.41±7.1** (38)	130.0±8.2 (9)	112.1±4.8** (41)	119.0±4.66 (6.3)
NH_4	148.6±4.4	139.7±6.5	115.3±7.2 (23)	135.5±4.1 (11)	112.5±6.9* (24)	123.3±7.2 (9)	87.0±2.8*** (41)	96.2±3.12 (10.3)
I_2	166.4±5.5 (-12.3)	135.0±4.9 -	117.2±17.7 (-4)	133.3±2.6 (-1.6)	121.2±3.2 (1.3)	121.36±4.5 (-6.9)	125.9±3.8 (11.6)	102.5±6.2 (-14)
I_4	198.5±6.2 (33)	119.2±6.0 -	119.2±5.9 (3.5)	132.8±3.3 -	158.1±8.7* (41)	121.96±3.6 (-1.0)	171.4±3.3*** (96)	94.9±2.5 (-1.4)
P_2	164.7±8.1 (13)	120.7±5.1 (8)	131.7±2.6 (8)	157.8±11.2 (17)	143.9±10.1 (21)	158.0±2.9 (22)	124.6±3.6 (12)	171.4±5.7*** (44)
P_4	251.9±4.1*** (69.5)	182.8±2.9*** (30)	156.9±8.1*** (36)	199.5±1.5*** (50)	145.6±4.2 (57)	158.1±11.1 (29)	123.2±5.3*** (41)	117.3±1.7*** (22)
IP_2	124.0±4.3 (35)	119.6±6.2 -	138.3±4.5 (13)	80.2±2.5 (-41)	187.8±2.4*** (57)	110.91±6.4 (-16)	123.2±4.1 (9.8)	105.5±7.2 (-11)
IP_4	152.1±7.1 (2.3)	126.6±8.1 -	135.9±5.3 (18)	123.7±9.1 (-7.0)	124.5±3.0 (11)	129.3±4.6 (4.9)	122.3±3.9*** (40.2)	97.2±6.6 -

Incubation of explants in insulin containing medium failed to enhance the rediolabelled ^{3}H-thymidine incorporation on day 2 in the normal control group, 40% and 60% parasitized liverand on day 2, 4 in the 20% parasitized liver. At 40% and 60% infection the incorporation increased by about 41% ($NH_4^{40\%}$ Vs $I_4^{40\%}$) and 96% ($NH_4^{60\%}$ Vs $I_4^{60\%}$) on the fourth day of culture as compared with the parasitized no hormone group (Figure 16).

Picroliv enhanced the incorporation of radioligand by about 8%, 36% at 20% parasitaemia ($NH^{20\%}$ Vs $P^{20\%}$); 21%, 29% at 40% parasitaemia ($NH^{40\%}$ Vs $P^{40\%}$) and 12%, 41% at 60% parasitized ($NH^{60\%}$ Vs $P^{60\%}$) in the liver explants on day 2 and 4 of cultured group as compared to respective parasitized control (Figure 18). After the incubation of explants in IP containing medium the increment was observed about 13%, 18% at 20% parasitaemia, 57%, 11% at 40% and 10%, 40% at 60% parasitaemia on day 2 and 4 of culture period (Figure 20).

^{3}H-Thymidine incorporation in Liver explants following Chloroquine (CQ) and Picroliv treatment: CQ treatment failed to affect the rate of radiotracer incorporation in either the normal controls, parasitized group at different parasitaemia levels on either days of culture of explants (Figure 15). Similar results were noticed in the explaints in insulin (I) (Figure 17) and insulin plus picroliv (IP) (Figure 21) containing medium. However, picroliv addition to the medium gave results which suggested some enhancement of the rate of incorporation of the radioligand i.e. 17% and 50% at 20% parasitaemia, 22% and 29% at 40% parasitaemia and 44% and 22% at 60% parasitaemia on day 2 and 4 respectively in each case (Figure 19).

3.2. In Spleen

3.2.1. Regulation of DNA Content by Chloroquine (CQ) and Picroliv Following P. berghei Infection

Administration of CQ to the parasitized animals and the subsequent culture of spleen explants resulted in the return to the normal values in terms of DNA content at different parasitaemia levels. In the spleen explants cultured from 20% parasitized animals, the reduction in DNA content was about 16%; at 40% infection 13%; and at 60% the level was 11% on the 0 and 3rd day of incubation when compared with the values from CQ untreated respective controls (Figure 22, Table 4).

Table 4. *Total DNA content in P. berghei infected murine spleen explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

Experiment	Control	Control+ CQ	20% parasitemia	20% parasitemia+ CQ	40% parasitemia	40% parasitemia+ CQ	60% parasitemia	60% parasitemia+ CQ
NH_0	60.9±8.2	100.7±11.1 (64)	82.2±3.7 (35)	69.2±2.9 (16)	91.11±7.6 (50)	79.22±7.6* (13)	103.03±6.1 (13)	92.1±6.7 (11)
NH_3	19.2±2.6	32.8±2.6 (71)	23.7±1.5 (23)	20.2±5.0 (16)	30.5±7.1 (59)	27.4±3.3 (13)	36.1±4.2* (88)	32.0±3.2 (11)
I_3	16.9±3.2 (12)	20.4±3.1 (38)	10.1±1.9 (57)	47.1±2.8* (-133)	29.5±59 (3.3)	49.9±6.4 (-82)	30.01±8.1 (17)	55.2±3.0 (-72)
P_3	50.2±2.5*** (-162)	20.4±2.2 (38)	18.9±2.1 (20)	1.2±0.5 (94)	21.5±5.1 (30)	12.3±3.3 (53)	24.2±2.4 (34)	20.0±2.9 (38)
IP_3	56.6±2.8 (-195)	12.8±1.9 (61)	28.7±3.3 (-21)	15.5±3.1 (-21)	39.9±2.3 (-31)	30.2±5.3 (-10)	47.01±3.7 (-31)	42.22±2.2 (-33)

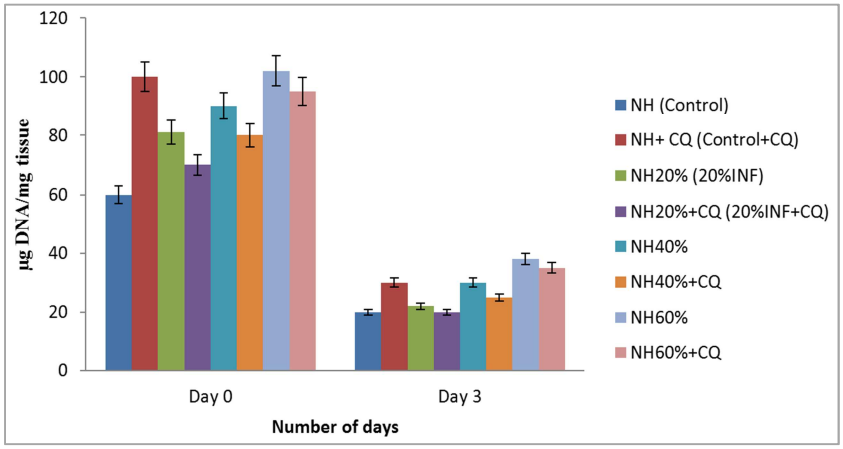

Figure 22. *Total DNA content in P. berghei infected murine spleen explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

After the addition of insulin in the medium, DNA content increased by 133% ($NH_3^{20\%}$ Vs $I_3^{20\%}$); 82% ($NH_3^{40\%}$ Vs $I_3^{40\%}$); and 72% ($NH_3^{60\%}$ Vs $I_3^{60\%}$) at 20%, 40% and 60% infections respectively as compared to the respective controls (Figure 23).

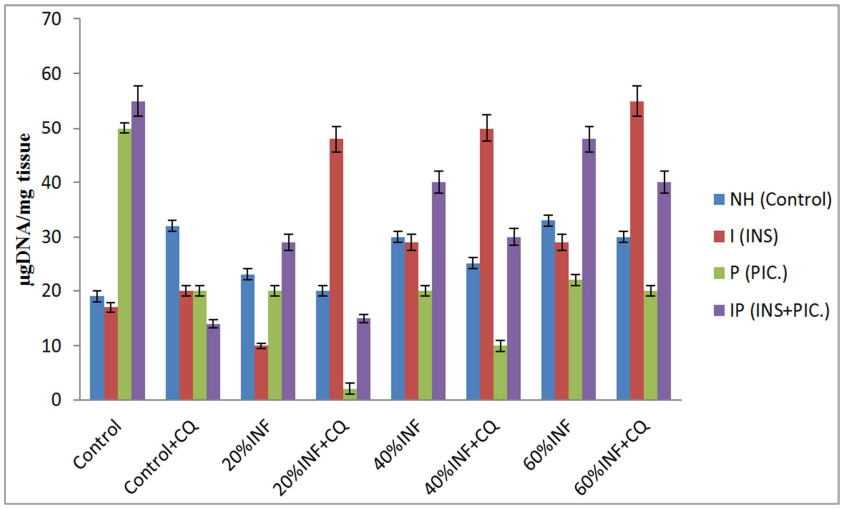

Figure 23. *In vitro effect of Insulin (INS, 1µg/ml), Picroliv (PIC, 0.5mg/ml) added separately & together on total DNA content in P. berghei infected, Chloroquine (CQ) treated, murine spleen explants cultured in serum-free medium at different parasitemia levels on Day3.*

After the incubation of explants in picroliv containing medium DNA values decreased by about 94% ($NH_3^{20\%}$ Vs $P_3^{20\%}$); 53% ($NH_3^{40\%}$ Vs $P_3^{40\%}$) and 38% ($NH_3^{60\%}$ Vs $P_3^{60\%}$) at the three levels of infection tested on day 3 of incubation as compared with CQ treated control group (Figure 23).

Presence of IP in the medium decreased the DNA index by about 61% in the normal control (NH_3^{C} Vs IP_3^{C}), 23% at 20% parasitaemia whereas at 40% and 60% infection DNA content increased by about 10% and 33% respectively (Figure 23).

3.2.2. Changes in Protein Turnover During P. berghei Infection

During *P. berghei* infection, in addition to the DNA content, the total protein content in the cultured tissue also increases with the increase in parasitaemia (Figure 24). Increase in the protein content was 43%, 17% at 20%, 68%, 56% at 40%; and 131% and 94% at 60% infection on day 0 and 3 of culture period as compared with the normal control group (Table 5).

Table 5. *Protein content in P. berghei infected murine spleen explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

Experiment	Control	Control+CQ	20% P	20% P+CQ	40% P	40% P+CQ	60%P	60%P+CQ
NH_0	1.9±0.31	1.03±0.22	2.72±0.03 (43)	2.1±0.04 (43)	3.2±0.03 (68)	2.5±0.51 (22)	$4.4±0.23^{**}$ (131)	3.1±0.04 (29.5)
NH_3	0.72±0.03	0.25±0.06	0.84±0.03 (17)	$0.29±0.06^{**}$ (66)	$1.12±0.05^{***}$ (56)	1.01±0.09 (10)	$1.4±0.04^{***}$ (94)	1.3±0.029 (7.1)
I_3	$1.2±0.06^{***}$ (66)	0.58±0.06 (-132)	0.62±0.09 (26)	$1.02±0.06^{**}$ (-252)	1.02±0.078 (8.9)	1.7±0.07 (-68)	1.9±0.01 (-36)	$2.2±0.038^{***}$ (-47)
P_5	$1.6±0.09^{***}$ (122)	$1.4±0.08^{***}$ (-460)	$0.35±0.081^{**}$ (58)	$1.1±0.05^{***}$ (-279)	1.10±0.045 (1.8)	1.1±0.027 (-9)	1.45±0.01 (-3.6)	$1.72±0.027^{**}$ (-15)
IP_3	$1.2±0.10^{*}$ (67)	$1.3±0.13^{***}$ (-420)	$0.37±0.09^{*}$ (56)	0.44±0.043 (-52)	$0.44±0.02^{***}$ (60)	1.0±0.03 (1)	$0.99±0.044^{**}$ (29)	1.51±0.05

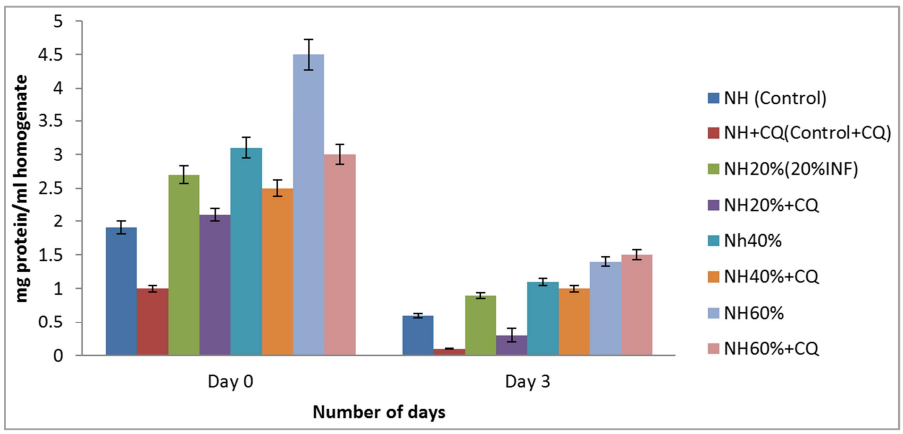

Figure 24. *Protein content in P. berghei infected murine spleen explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

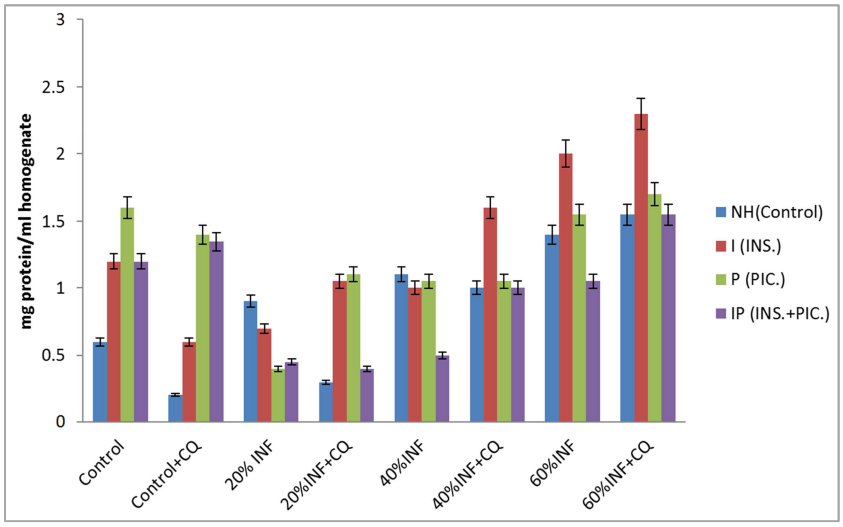

Figure 25. *In vitro effect of Insulin (INS, 1µg/ml), Picroliv (PIC, 0.5mg/ml) added separately & together on Protein content in P. berghei infected, Chloroquine (CQ) treated, murine spleen explants cultured in serum-free medium at different parasitemia levels on Day3.*

Supplementation of insulin in the culture medium attenuated the protein content by about 26% at 20% parasitaemia ($NH_3^{20\%}$ Vs $I_3^{20\%}$); 8.9% at 40% parasitaemia ($NH_3^{40\%}$ Vs $I_3^{40\%}$) on day 3 whereas at 60% parasitaemia insulin increased the protein content by about 36% ($NH_3^{60\%}$ Vs $I_3^{60\&}$) as compared to respective control (Figure 25).

Addition of picroliv decreased the protein values by about 58% at 20% parasitaemia ($NH_3^{20\%}$ Vs P_3^{20}) whereas at 40% and 60% infection picrolive was almost failed to alter the protein content in comparison with respective controls (Figure 25).

Incubation of explants in IP containing medium decreased the protein content by about 56% ($NH_3^{20\%}$ Vs IP_3^{20}), 60% ($NH_3^{40\%}$ Vs $IP_3^{40\%}$); and 29% ($NH_3^{60\%}$ Vs $IP_3^{60\%}$) at 20%, 40% and 60% parasitaemia on day 3 of culture as compared with the NH group (Figure 25).

Protein turnover during P. berghei infection after Chloroquine (CQ) and Picroliv treatment: After the CQ treatment, protein content decreased by 23%, 66% at 20% parasitaemia, 22%, 10% at 40% parasitaemia and 30%, 7% at 60% parasitized group on day 0 and 3 respectively (Figure 24). Addition of insulin to the culture medium increased the protein content by about 132% in normal control group (NH_3^C Vs I_3^C); 252% at 20% parasitaemia ($NH_3^{20\%}$ Vs $I_3^{20\%}$); 68% at 40% parasitaemia ($NH_3^{40\%}$ Vs $I_3^{40\%}$) and 47% at 60% parasitaemia ($NH_3^{60\%}$ Vs $I_3^{60\%}$) on day 3 as compared to their controls (Figure 25).

Incubation of explants in Picroliv supplemented medium results in an increase of 460% in the normal control (NH_3^C Vs P_3^C), 279% at 20% parasitaemia, 9% at 40% parasitaemia and 15% at 60% parasitaemia levels on day 3 ($NH_3^{Inf.}$ Vs $P_3^{Inf.}$) (Figure 25).

Similar pattern was noticed with the supplementation of IP in the medium. In the normal control about 420% (NH_3^C Vs I_3^C); and 20% parasitaemia about 52% ($NH_3^{20\%}$ Vs $IP_3^{20\%}$) increase was observed on day 3 as compared to NH_3 group of the respective controls. No effect was observed at 40% and 60% infection (Figure 25).

3.2.3. ^{3}H-Thymidine Incorporation in the Spleen Explants During P. berghei Infection

During *P. berghei* infection, extend of ^{3}H-Thymidine incorporation increased with increasing parasitaemia (Figure 26). At 20% parasitaemia the incorporation of radioligand increased by about 3.4% (NH_0^C Vs $NH_0^{20\%}$) on day 0 and 8.4% (NH_3^C Vs $NH_3^{20\%}$) on day 3 of culture. At 40% parasitaemia increase in ^{3}H-Thymidine incorporation was 14%, 13.3% on day 0 and 3 respectively (NH^C Vs $NH^{40\%}$) and at 60% parasitaemia about 21.5%, 20% increase was noticed on day 0 and 3 (Table 6).

Table 6. *3H-Thymidine incorporation in P. berghei infected murine spleen explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

Experiment	Control	Control+ CQ	20% parasitemia	20% parasitemia+ CQ	40% parasitemia	40% parasitemia+ CQ	60% parasitemia	60% parasitemia+ CQ
NH_0	314.58±4.2	321.85±2.2 (2.3)	325.78±4.6 (3.4)	318.44±1.9 (2.09)	358.8±4.4* (14)	329.99±4.9 (8.8)	382.2±3.2*** (21.5)	349.92±9.1**
NH_3	294.11±2.3	301.22±3.7 (2.42)	318.82±4.2* (8.4)	299.29±2.2 (6.13)	333.33±3.5*** (13)	301.01±3.1** (9.7)	353.30±4.1*** (20.2)	322.21±3.1** (8.8)
I_3	292.11±5.1 (0.7)	299.11±7.3 -	280.21±1.8** (12.11)	321.21±3.1** (-7.3)	291.19±2.1*** (12.65)	344.22±5.1** (-14.4)	307.22±3.9** (13.05)	362.24±5.0* (-12.4)
P_3	333.21±1.9*** (-13.3)	29.29±6.1 -	278.84±7.1** (12.5)	210.12±3.9*** (30)	281.42±1.8*** (15.6)	250.2±2.1*** (16.9)	299.92±33.3*** (15.1)	275.57±5.3** (14.5)
IP_3	350.22±5.5*** (-19)	210.36±4.4 (30)	310.68±4.4 (2.6)	262.32±5.1** (12.4)	328.88±4.7 (1.3)	318.18±1.6* (-5.7)	337.75±7.2 (4.4)	323.23±2.2 -

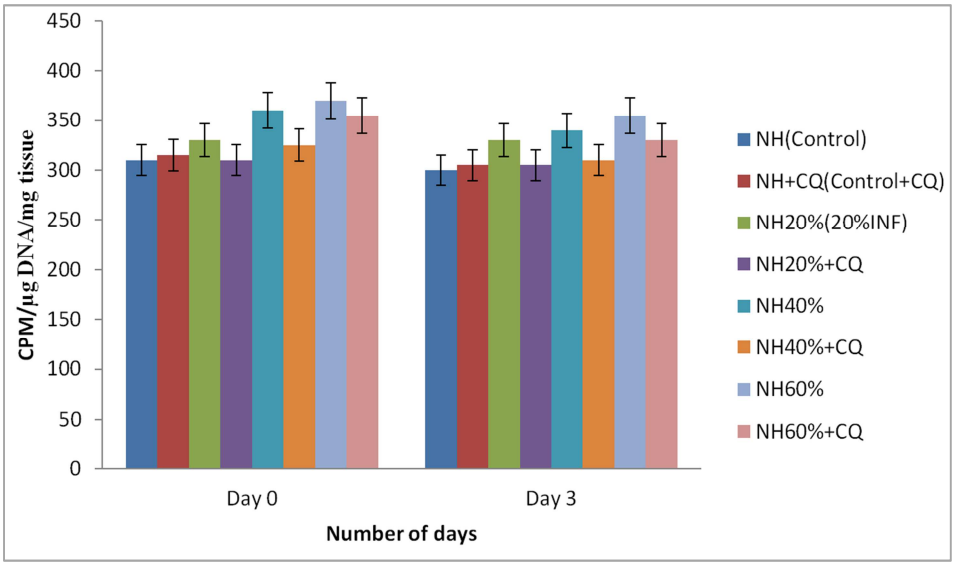

Figure 26. *3H-Thymidine incorporation in P. berghei infected murine spleen explants cultured in serum-free medium at different parasitemia levels with Chloroquine (CQ) treatment.*

Extraneously added insulin decreased the ^{3}H-thymidine incorporation by about 0.7% in controls (NH_3^C Vs I_3^C); 12% at 20% parasitaemia ($NH_3^{20\%}$ Vs $I_3^{20\%}$); 12.7% at 40% parasitaemia ($NH_3^{40\%}$ Vs $I_3^{40\%}$); and 13% at 60% parasitaemia ($NH_3^{60\%}$ Vs $I_3^{60\%}$) on day 3 of incubation.

Upon incubation of explants in Picroliv containing medium, about 13.3% increase was observed in the normal control group (NH_3^C Vs P_3^C). On the other hand about 12.5%, 15.6% and 15.1% decrement was noticed on 20%, 40% and 60% parasitaemia level on day 3 of culture (NH Vs P).

Addition of IP in the culture medium demonstrated similar pattern. In the normal control group, about 19% increment was noticed. Conversely, in the infected group 2.6%, 1.3% and 4.4% decrease was observed on day 3 of culture at 20%, 40% and 60% parasitaemia levels respectively.

3.2.4. ^{3}H-Thymidine Incorporation in the Spleen Explants After Chloroquine (CQ) and Picroliv Treatment

After CQ treatment, about 2.1%, 6.13% decrement was observed at 20% parasitaemia on day 0 and 3 of incubation as compared to CQ untreated respective controls. At 40% parasitaemia, about 8.8% and 9.7% decrement and at 60% infection 8.5% and 8.7% decrement was observed on day 0 and 3 of culture comparatively with CQ untreated respective control groups (Figure 26).

Supplementation of insulin increased ^{3}H-thymidine incorporation about 7.3% at 20% parasitaemia ($NH_3^{20\%}$ Vs $I_3^{20\%}$); 14.4% at 40% parasitaemia ($NH_3^{40\%}$ Vs $I_3^{40\%}$); and 12.4% at 60% parasitaemia ($NH_3^{60\%}$) Vs $I_3^{60\%}$).

Addition of picroliv in the culture medium decreased the radioligand incorporation by about 30% at 20% parasitaemia ($NH_3^{20\%}$ Vs $P_3^{20\%}$); 16.9% at 40% parasitaemia ($NH_3^{40\%}$ Vs $P_3^{40\%}$); and 14.5% at 60% parasitaemia ($NH_3^{60\%}$ Vs $P_3^{60\%}$).

Supplementation of IP altered the incorporation of radioligand by about 12.4% at 20% parasitaemia ($NH_3^{20\%}$ Vs $IP_3^{20\%}$); and 5.7% at 40% parasitaemia ($NH_3^{40\%}$ Vs $IP_3^{20\%}$) whereas at 60% parasitaemia no significant change was noticed when compared with the respective controls.

4. Discussion

4.1. DNA and Protein Turnover in Liver and Spleen Explants in Culture During P. berghei Infection

It has been observed that DNA and Total Protein content undergo significant alterations during *P. berghei* infection in the liver (Tables 1 & 2) and spleen explants in culture. In the liver, the DNA and protein content decreases with the advancement of parasitaemia (Figures 1 & 8). Conversely, in the spleen explants, the levels of DNA and protein increase with rising malarial infection. These results have also been supported by several workers [18]. It is well known that purines are synthesized by salvage pathways in mammals (Phillips, 1984). Thus, the malarial parasites are dependent upon salvage pathways of the host due to their inherent incapability to synthesize nucleotides *de novo*.

Nucleotide uptake by the parasites from the host under such conditions might be one of the reasons for the reduction of nucleic acids in the liver during malarial infection. Thus, the reduction in nucleotide content may be expected to reduce the DNA and the concomitant protein synthesis of the host liver during *P. berghei* infection. Von Brand (1973) has also interpreted these findings and described that the malaria parasites utilize the same 20 amino acids, used in the protein synthesis in mammals, for their own protein synthesis. The continuous extensive proteolysis provides a readily available pool of free amino acids to the parasites for their rapid proliferation.

Inhibition of protein synthesis in the host liver as a consequence of *P. berghei* infection has been confirmed by *in vitro* studies. It have described that the distintegrated Rough Endoplasmic Reticulum (RER) may be one of the causes of the inhibition of protein synthesis during *P. berghei* infection [19].

On the other hand, the DNA and protein content were

found to increase in the spleen during malarial infection (Figures 22 & 24). Presence of phagocytosed, infected red blood cells, parasites and immunologically active splenocytes might be the cause of elevated protein level in the spleen. In the post CQ treated parasitized liver, a significant increment of the DNA and protein levels was observed (Figures 1 & 8). Conversely, in the case of spleen under similar conditions, decreased DNA and protein level were obtained as compared to respective CQ untreated group (Figures 22 & 24). It may be surmised from the foregoing that due to the blood schizonticidal activity of CQ, the consumption of DNA and protein by the parasites decreases causing reversal in the level after CQ treatment.

Insulin supplementation to the medium containing CQ untreated liver explants demonstrates a promotion in the DNA level in both the normal control and the 20% parasitized group (Figure 2). Similar increasing pattern was obtained for protein turnover except in the normal and 20% parasitized liver in which insulin failed to exert its effect (Figure 9). The effect of insulin was noticeable with the advancement of parasitaemia. In the CQ treated liver explants, insulin failed to enhance the DNA and protein turnover during the entire culture period (Figures 3 & 10). Conversely, in the case of CQ untreated infected spleen explants, cultured in the presence of insulin, a significant decrement was noticed. After the CQ treatment of infected animals and culture of spleen explants in presence of insulin enhanced DNA turnover was observed (Figure 23).

Addition of picroliv to the culture medium resulted in the restoration of DNA and protein contents to normal levels in the parasitized untreated and 60% infected CQ treated liver (Figures 4, 5, 10 & 11) and parasitized untreated spleen (Figures 23 & 25). In the spleen derived from CQ treated parasitized animals, picroliv failed to alter the protein and DNA turnover (Figure 24). Similar profile was observed in parasitized liver explants cultured in IP containing medium (Figures 6, 7, 12 & 13).

4.2. Role of Insulin During P. berghei Induced Alterations in Liver and Spleen Explants

Insulin is an essential component involved in the overall process of cell proliferation of diverse types of cells. According to Tappy and Acheson (1998) insulin stimulates and controls the glucose oxidation and transportation in various tissues. In the present study, insulin represents a positive regulator of histoarchitectural and biochemical mechanisms in the absence of CQ treatment. In the liver derived from parasitized mice reduction in the extent of pigmentation, narrowing of the inter-sinusoidal space and normal size of nuclei was observed. After the culture of infected spleen explants in insulin containing medium, a reduction in the extent of hemozoin and hemosiderin deposition was observed. The outer capsular wall, trabeculae and overpopulation of spleen cells acquire normotypical appearance. On an overall basis, insulin causing significant upregulation in the enzyme systems may be due to its central role and overall governance of cellular homeostasis. During the last few years, evidence has also accumulated for the direct acute effect of insulin on lipid and glycogen metabolism using liver system *in vitro*. Insulin can stimulate the incorporation of ^{3}H-thymidine justifying its dominant role in promoting hepatic DNA synthesis [20]. Role of insulin in the processing of amino acids, hexose transport, glycogen and fatty acid synthesis has been described.

In the CQ treated parasitized animals, insulin demonstrates the adverse effect on the histo-architecture and biochemical parameters of tissues. No effect was found on the pigmentation/vacuolation of cells. Black coloured patches were observed on the liver with the advancement of infection. The capsular wall of the spleen was found to be ruptured and no effect was found on the congestion of cell population. Several workers have observed the effect of quinine with insulin and glucose also attributed the hyperinsulinaemic effect of quinine [21].

4.3. Hepato-protective and Immuno-stimulant Activity of Picroliv

Picroliv, an irridoid glycoside isolated from the roots and rhizomes of *Picrorhiza kurroa,* shows a pronounced protective effect against liver and spleen damage caused by *Plasmodium berghei* in mice. Picroliv, present in culture medium at a concentration of 0.5 mg/ml, restored the histo-architectural changes in liver and spleen explants induced during *P. berghei* infection. The architecture of the infected liver appeared typical with the normal cytoplasmic vacuolation and absence of bi nucleated parenchymal cells. In addition, the absence of malarial pigments (up to 40% parasitaemia) with the normal sinusoidal space was also noticed. Additionally, picroliv treatment also provided favourable conditions for the spleen regeneration to the normal status. The histo-morphomatrices of the parasitized spleen explants appeared typical with the absence of malarial pigments. Withdrawal of over congestion and decreased inflammation of the capsular wall further support the overall protective effect of picroliv.

Extraneously supplemented picroliv reversed the changes in DNA, RNA and protein content of liver and spleen explants induced during *P. berghei* infection. Presence of picroliv in the medium stimulates the DNA and protein synthesis. G_1 phase of cell cycle was found to progress in picroliv treated regenerative liver RNA and protein synthesis is also commensurate with the DNA synthesis [22].

As reported earlier in this dissertation, the extent of protein synthesis undergoes alteration during *P. berghei* infection and the basic mechanism of liver damage is due to the centrally exerted inhibition of DNA turnover. Picroliv addition to the medium prevents the inhibition of protein sysnthesis in a site-specific manner against several agents affecting transcription or translation. Several workers have observed the mechanisms of various inhibitory agents of protein synthesis and also described the mode of action of picroliv in preventing the inhibition of protein synthesis. Actinomycin-D (a protein synthesis inhibitor) binds tightly and specifically to DNA by intercalating between neighboring guanine-

cytosine (G-C) base pairs of the double helix, preventing DNA from being an effective template for RNA synthesis. According to them, picroliv may prevent the DNA-actiomycin-D interaction by interfering with the binding of actiomycin-D between the G-C pairs of the double helical DNA [23].

Puromycin acts at the translational level and binds to the A site on the ribosome and inhibits entry of incoming aminoacy[1] t-RNA. The growing polypeptide chain gets covalently bound to puromycin to form peptidy[1]-puromycin and thus causes premature release of incomplete polypeptide chain. In this case, picroliv can attach itself at the A site and thus block the entry of puromycin. It may form a complex with the active binding site of puromycin, rendering it inactive. The protective effect of picroliv thus appears to be site-specific. Dwivedi *et al* (1991) have also reported the protective effect of picroliv against hepatotoxicity induced by aflatoxin B_1 and *Amanita phalloids* in rts. Both of them inhibit the protein biosynthesis at the transcriptional level by blocking DNA directed RNA polymerase II. These results provide explanation for the ability of picroliv to prevent the inhibition of protein synthesis in a site specific manner against agents affecting transcription and translation thereby playing a major role in its hepato-protective activity.

In summary, our studies have led to the enunciation of a serum-free culture system with which it is possible to quantify the acute and chronic changes that the tissue can undergo under normal and parasitic conditions. In addition, it also permits us to experiment with an *in vitro* test system for evaluating the efficacy of antimalarial compounds on a long term basis unlike the traditional the traditional *in vivo* approaches that have been employed so far.

5. Conclusion

Total DNA content was observed to decrease as the arasitemia level increases. After addition of insulin, could increase the DNA content slightly, this was further enhanced by application of picroliv in the media. A similar positive effect of picroliv was also found in case of total protein content and 3-H-Thymidine incorporation in both the case of liver and spleen explants culture.

Author's Contribution

Esha Sarkar, Anchal Trivedi carried out all the laboratory work as well as contributed in researching the data for this article, writing the article content. Aparna Misra and Anil Kumar Balapure helped with reviewing and editing of the manuscript before submission and during the revision process. All the authors read and approved the final manuscript.

Ethics Approval and Consent to Participants

Not applicable.

Human and Animal Rights

No animals/humans were used for studies that are the basis of this research.

Consent for Publication

Not applicable.

Availability of Data and Materials

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Conflict of Interest

The authors declare no conflict of interest.

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