International journal of basic and applied research
www.pragatipublication.com
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.960

Evaluation of biochemical changes in Plasmodium berghei infected mice treated with Artemisinin-based combination therapy

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Received: 10 April Revised: 18 April Accepted: 26 April

Abstract

Background: Malaria pathogenesis is based mainly on extensive changes in biochemical parameters. Thus the present investigation was aimed to study the changes in biochemical parameters in Plasmodium berghei infected mice treated with artemisinin-based combination drugs i.e., Artesunate + Amodiaquine (ASAQ), Artesunate + Sulfadoxine Pyrimethamine (ASSP), (Artesunate + Lumefantrine (AL) on the biochemical parameters of P. berghei infected mice.
Methods: The course of infection was observed in P. berghei infected mice and drug treated experimental mice by observation of blood smear for malaria parasite stages. The biochemical parameters were estimated by following the standard methods.
Results: The blood glucose content was decreased in P. berghei infected mice which resulted in hypoglycemia; total protein and albumin contents were decreased whereas globulin content was increased in infected mice; total bilirubin, direct and indirect bilirubin contents increased in infected mice, the enzymes AST, ALT and ALP were also increased in infected mice significantly when compared to the control mice without P. berghei infection. But when these infected mice were treated with ASAQ, ASSP and AL, all these biochemical parameters were restored to normal level. Amongst these drug combinations, ASAQ combination was the most efficient than ASSP and AL.
Conclusion: The artemisinin-based combination drugs are recently being used for treatment malaria because of growing drug resistance. Hence we studied the above drug combinations, but ASAQ was found to be most efficacious than ASSP and AL in treating P. berghei infection and restoring the biochemical parameters.

Key words: Plasmodium berghei, Biochemical parameters, Artemisinin-based combination drugs .

Introduction

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Malaria is a global disease that is predominant in the tropics and caused by blood parasites (a type of single cell microorganism of the Plasmodium). Malaria symptoms typically include fever, fatigue, vomiting and headaches. In severe cases, it can cause yellow skin, seizures, coma or death. These symptoms usually begin ten to fifteen days after being infected. In those who have not been properly treated, disease may recur months later. In those who have recently survived an infection, re-infection typically causes milder symptoms. This partial resistance disappears over months to years if there is no ongoing exposure to malaria (Caraballo, 2014).

Malaria-associated biochemical parameter alteration and organ dysfunction is one of the major life-threatening causes of death occurs in 5% - 10% of hospitalized patients (Mehndiratta, 2013). The malaria-associated hypoglycemia and alteration of biochemical parameters includes blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin and bilirubin etc.

Liver destruction can affect the metabolic processes in the body due to the role of liver in general metabolism of the organism. Enzymes are necessary for normal cellular metabolism including that of the liver (Rajamanickam and Muthuswamy, 2008). When the infected anopheline mosquito takes a blood meal, sporozoites are inoculated into the blood stream. Within an hour, sporozoites enter hepatocytes and begin to divide into exoerythrocytic merozoites (tissue schizogony) (Trampuz et al., 2003). Once merozoites leave the liver, they invade erythrocytes. In return, malaria parasites affect the liver. Alanine and aspartate transminase (ALT and AST) activities are used as indicators of hepatocytes damage (Asagba et al., 2004; Coppo et al., 2002; Dede et al., 2002; Whitehead et al., 1999). Akanji et al. (1993) reported that alkaline phosphatase (ALP) is a marker enzyme for the plasma membrane and endoplasmic reticulum.

Plasmodium berghei has been used in studying the activity of potential antimalarials in mice (Pedroni et al., 2006) and in rats (English et al., 1996). It produces diseases similar to those of human Plasmodium infection (Kumar et al., 2006 and Peter and Anatoli, 1998). Plasmodium berghei has a very similar life cycle to the species that infect humans, and it causes disease in mice which have signs similar to those seen in human malaria (David et al., 2004). Studies have also shown that haematological and biochemical parameters have been reported to be a reliable parameter for assessment of the health status of animals (Sexena et al., 2011; Ohaeri and Eluwa, 2011).

A resistant phenotype has been detected in five countries of The Greater Mekong Sub-region: Cambodia, the Lao People's Democratic Republic, Myanmar, Thailand and Vietnam, as relatively slow parasite clearance rates in patients receiving artemisinin or ACT (White, 2011; Cui et al., 2012; Phyo et al., 2012). And signs of resistance to the ACT-artesunate sulfadoxine pyrimethamine in Plasmodium falciparum have been observed in North-eastern states of India (Operational Manuel for Malaria Elimination in India, New Delhi: 2016).

There have been two case reports of artesunate-resistance in India, occurring in Kolkata and Mumbai (Bhattacharyya et al., 2014; Gogtay et al., 2000). There have been four cases of suspected artesunate –resistant malaria from Andhra Pradesh-Orissa border province in India. Out of the four cases, three cases were from our state Andhra Pradesh i.e., from Vizianagaram and Visakhapatnam districts (Shalini et al., 2018).

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Thus, the aim of the present study was to investigate the effect of some Artemisinin- based combination therapies (ACTs) on different biochemical parameters in P. berghei infected and treated experimental mice.

Materials and methods

Experimental Animals:

Thirty male Swiss albino mice each weighing 25-30 g were divided into 5 experimental groups each with 6 animals (n = 6). Animals were allowed to acclimatize for one week before initiation of the experiment. They were housed in plastic cages with rice husk as beddings, provided with access to commercial pellet food and access to clean drinking water ad libitum. The animals were handled in accordance with the guidelines in the Guide for the care and use of laboratory animals (2011). Animal experiments were designated and approved with Ref. No. ANUCPS/IAEC/AH/Protocol/2/2014 by Institutional Animal Ethics Committee (IAEC) of ANU College of Pharmacy, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Parasite:

Chloroquine sensitive P. berghei ANKA strain parasites were maintained by intraperitoneal inoculation of 1×10^7 infected erythrocytes to naïve mice. A standard inoculum consisting of 1×10^7 parasitized erythrocytes was prepared from the infected donor mice with >25% parasitaemia, and used to infect experimental mice.

Inoculation of Experimental Animals:

Parasitized red blood cells used for inoculation were obtained by cardiac puncture from a donor mouse. The infected blood was collected in an anticoagulant and diluted to the desired density in 0.9% normal saline. Each mouse was inoculated with 1×10^7 parasitized red blood cells of P. berghei suspension. The infection of the recipient mice were initiated by needle passage of the parasite preparation from the donor to healthy test animals via the intraperitoneal route as described previously (David et al., 2004). The day of inoculation was defined as Day 0 and subsequent days as Day 1, Day 2, and Day 3 up to Day 28.

Drugs and Dosage Regimens:

In the present work, three Artemisinin-based combination drugs were used namely Atesunate + Amodiaquine (AS+AQ), Artesunate + Sulphadoxine Pyrimethamine (AS+SP), Artemether + Lumefantrine (AL). All the drug dosages were given according to the body weight of mouse by following standards of World Health Organization (WHO).

(i) Artesunate+Amodiaquine (ASAQ):

The combination drugs of Artesunate (50 mg) tablet and Amodiaquine Hydrochloride (153.1 mg) tablet from IPCA Laboratories Limited, Mumbai. Artesunate (50 mg) tablet was dissolved in 50 ml of distilled water to obtain the stock solution concentration of 1 mg/ml. And 153.1 mg tablet of Amodiaquine dissolved in 150 ml of distilled water to obtain the stock solution concentration of 1.02 mg/ml. The WHO dosage regimen is Artesunate 4 mg/kg + Amodiaquine 10 mg/kg once a day for 3

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days. So in the present experiment, the same WHO recommended dosage regimen was followed and administered to the infected mice for 3 days by oral gavage according to the body weight.

(ii) Artesunate + Sulphadoxine Pyrimethamine (ASSP):

The combination drugs of Artesunate (200 mg) tablet and Pyrimethamine (25 mg) + Suphadoxine (500 mg) tablet (LARINATE-200 kit) from IPCA Laboratories Limited, Mumbai. Artesunate stock solution (1 mg/ml) was prepared as was in 2.4.1. And Pyrimethamine (25 mg) + Sulphadoxine (500 mg) tablet was dissolved in 100 ml of distilled water to obtain the stock solution concentration of 5.25 mg/ml. The WHO dosage regimen is Artesunate 4 mg/kg once daily for 3 days and Sulphadoxine + Pyrimethamine as single dose of 25 mg/kg + 1.25 mg/kg on Day 1, which was administered orally. The above WHO dosage regimen was followed in the present experiment.

(iii) Artemether+Lumefantrine (AL):

The third combination drug used was Artemether (20 mg) and Lumefantrine (120 mg) tablet (LUMERAX-20 DT) from IPCA Laboratories Limited, Mumbai, India. The tablet Artesunate (20 mg) and Lumefantrine (120 mg) was dissolved in 50 ml of distilled water to obtain the stock solution concentration of 2.8 mg/ml respectively. The WHO dosage regimen is Artemether 1.5 mg/kg and Lumefantrine 9 mg/kg at 0, 8, 24, 36, 48 and 60 hour. The same WHO regimen was followed and 6 doses were given on 3 consecutive days.

A combination therapy would be helpful in simultaneous use of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite (WHO, 2001).

Animal Groups:

The mice were divided into following 5 groups with 6 mice (n = 6) in each group:
Group 1 (Control): The mice were given only distilled water.
Group 2 (Infected Non-treated): The mice were infected with P. berghei antigen.
Group 3 (Infected + ASAQ): The mice were first infected with P. berghei antigen and then treated with Artesunate+Amodiaquine combination.
Group 4 (Infected +ASSP): The mice were first infected with P. berghei antigen and then treated with Artesunate + Sulphadoxine Pyrimethamine combination.
Group 5 (Infected + AL): The mice initially were parasitized with P. berghei and then treated with Artemether + Lumefantrine combination.

Study of the course of infection in Plasmodium berghei infected mice:

Thin blood films were prepared on clean slides, initially fixed with methanol. A large drop of blood is put at the center of a clean dry slide. The drop is spread with an applicator slide, and then the smear is thoroughly dried in a horizontal position. Blood smears were stained with Giemsa stain for 5-8 min. Subsequently, distilled water was poured on the surface of the smears to remove excess stain and then dried. A field was selected using x10 objective where the Red Blood Corpuscles (RBCs) were in an evenly distributed monolayer followed by the x100 oil immersion objective. A minimum of 1000 RBCs were counted and among those, number of infected RBCs will be recorded. The percent of

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infected RBCs (parasitaemia) was determined by enumerating the number of infected RBCs in relation to the number of uninfected RBCs (Oyewole et al., 2010) as follows.

Percentage (%) of Parasitaemia = $\frac{\text{No. of infected RBCs}}{\text{No. of RBCs counted}} \times 100$

Collection of blood serum:

In all the experimental groups (both infected non-treated and infected- treated mice), parasitaemia was estimated throughout the experimental period daily by observation of Giemsa stained blood smears under the microscope. On 7th day of the of experimental period, the required number of mice were euthanized with chloroform and the blood samples were collected through cardiac puncture and serum samples were obtained for estimation of biochemical parameters.

Estimation of biochemical parameters:

The serum glucose levels were determined by Ortho-toluidine method (Mono-step). Total serum protein and albumin were determined by Biuret method (Kinsley,1972) and anionic bromocresol dye binding method respectively (Doumas and Biggs, 1972). Serum globulin was determined indirectly by difference between total serum protein and serum albumin (Watson, 1965). Serum bilirubin (total, direct and indirect) by diazo reaction method (Noslin, 1960). A/G ratio was caliculated by albumin/ globulin levels. Aspartate and alanine transaminases (AST & ALT) were estimated by colorimetric method (Reitman and Frankel, 1957). ALP was assayed by Phenolphthalein method (Babson et al.,1966).

Statistical analysis:

Results of individual parameters were expressed as mean ±standard deviation. The comparison between the experimental groups was performed by Student t-test using MINITAB 11.12.32 Bit statistical package and graphs were drawn in MS Excel. The results were statistically significant at P <0.001.

Results

Course of infection to P. berghei in experimental mice:

(i) P. berghei Infected Non-treated group:

During the study of course of infection, P. berghei parasite was given to the experimental mice on Day 0. After inoculation the parasitaemia was first appeared on Day 3(72hours).Then the parasitaemia was gradually increased up to the peak level on Day 7.On Day 3, initial parasitaemia was 19%, on Day 4 with 23%, on Day 5 with 27%, on Day 6 with 32% and on Day 7 with 36% of parasitaemia. High rate of parasitaemia was observed on 7th day post inoculation after which all the mice died due to heavy infection by Day 8 (Figure 1).

(ii) P. berghei Infected + ASAQ Treated:

In this group, initial parasitaemia was 20% on Day 3. On Day 3, Day 4 and Day 5; the therapeutic dose of ASAQ combination drug was administered orally. Then the parasitaemia was

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decreased to 8% on Day 4. On Day 5, the parasitaemia was 0% and so the parasite clearance occurred within 48 hours. No recrudescence was observed during the follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time (PCT) in ASAQ treated mice was 2 days (48 hours) (Figure 1).

(iii) P. berghei Infected + ASSP Treated:

In this group, the initial parasitaemia was 21% on Day 3. On Day 3, Day 4 and Day 5; the therapeutic dose of ASSP drug was administered orally. Then the parasitaemia decreased to 10% on Day 4. On Day 5, the parasitaemia was 2% and on Day 6 with 0%. No recrudescence was observed during follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time of (PCT) in ASSP treated mice was 3 days (72 hours) (Figure 1)

(iv) P. berghei Infected + AL Treated:

In this group, the initial parasitaemia was 19% on Day 3. Then the mice were treated with AL combination drug for 3 consecutive days orally on Day 3, Day 4 and Day 5. On Day 4 the parasitaemia was 11%, on Day 5 parasitaemia decreased to 3% and on Day 6 no parasitaemia was observed. Also no recrudescence was observed during the follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time (PCT) in AL treated mice was 3 days (72 hours) (Figure 1).

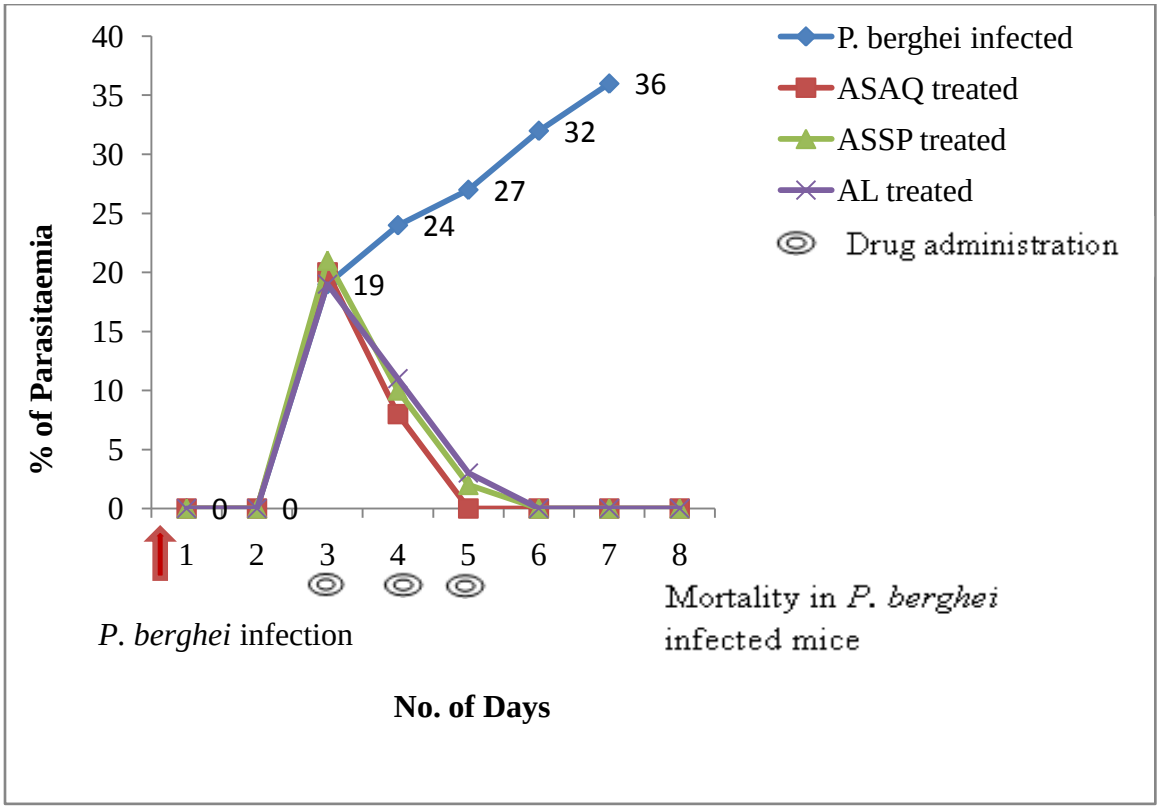

Figure 1: Course of infection to Plasmodium berghei in infected non-treated and treated mice during Artemisinin-based combination therapy

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Changes in biochemical parameters in different experimental mice:

In the present study, the changes in biochemical parameters were evaluated in control, P. berghei infected and three drug treated groups i.e., ASAQ, ASSP and AL treated mice.

The values of all the biochemical parameters of the experimental mice are represented in Table 1.

In P. berghei infected mice, the biochemical parameters were altered significantly (P <0.001) when compared to the control mice without P. berghei infection because of peak level of infection on 7th day.

The study revealed that blood glucose level was significantly decreased (P <0.001) in the P. berghei infected mice with hypoglycemia when compared to the control mice. But in ASAQ, ASSP and AL treated mice, the blood glucose levels were restored to normal levels as in control mice.

In the P. berghei infected group, the total protein and total albumin levels were significantly decreased (P <0.001) but globulin levels were significantly increased (P <0.001) when compared to the control group. After treatment with ASAQ, ASSP and AL treatment; total protein, albumin and globulin levels have shown normal values like in control group.

The total bilirubin, direct and indirect bilirubin contents were increased significantly (P <0.001) in P. berghei infected mice when compared to the control mice without infection. But when these infected mice were treated with ASAQ, ASSP and AL, the bilirubin contents were restored to normal.

The enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were significantly increased (P <0.001) in P. berghei infected group when compared to the control group. But after ASAQ, ASSP and AL treatment, these enzymatic levels were restored to normal levels.

So in treated groups, all the above biochemical parameters were restored to normal levels significantly (P <0.05) (Table 1). But amongst these three drug combinations, ASAQ combination was found to be the most effective than ASSP and AL in treating the mice by restoring all the biochemical parameters to normal levels.

Table 1: Changes in serum biochemical parameters in Plasmodium berghei infected non-treated and treated mice with Artemisinin-based combination drugs

S No.	Parameter	Control Non-infected (Normal) (n =6)	P. berghei Infected non-treated (n =6)	P. berghei Infected + ASAQ treated (n =6)	P. berghei Infected + ASSP treated (n =6)	P. berghei Infected + AL treated (n =6)
1	Blood Glucose (mg/dL)	194 ±1.41	85 ±0.913 P =0.0000* T =125.86	182 ±0.589 P =0.0000* t =178.59	179 ±0.365 P =0.0000* t =191.21	170 ±0.658 P =0.0000* t =151.05
2	Total	5.62 ±0.126	4.7 ±0.081	5.42 ±0.171	5.15 ±0.057	5.27 ±0.095

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	Protein (g/dL)		P =0.0000 T =12.33	P =0.0003* t =7.66	P =0.0001* t =9.00	P =0.0001* t =9.14
3	Total Albumin (A) (g/dL)	3.42 ±0.064	1.50 ±0.043 P =0.0000 T =49.27	3.3 3 ±0.111 P =0.0000* t =30.73	3.28 ±0.021 P =0.0000* t =73.00	3.11 ±3.118 P =0.0000* t =25.5
4	Total Globulin (G) (g/dL)	1.9 ±0.024	2.20 ±0.081 P =0.0004 T =7.04	1.84 ±0.017 P =0.0001* t =8.57	1.80 ±0.016 P =0.0001* t =9.61	1.90 ±0.020 P =0.0004* t =7.07
5	A/G Ratio	1.70 ±0.017	0.67 ±0.015 P =0.0000 T=109.75	1.78 ±0.018 P =0.0000* t =113.05	1.79 ±0.025 P =0.0000* t =91.52	1.76 ±0.020 P =0.0000* t =104.14
6	Total Bilirubin (mg/dL)	0.18 ±0.018	0.80 ±0.070 P =0.0000 T =17.17	0.19 ±0.029 P =0.0000* t = 6.10	0.02 ±0.071 P =0.0000* t =11.96	0.21 ±0.044 P =0.0000* t =14.24
7	Direct Bilurubin (mg/dL)	0.02 ±0.014	0.06 ±0.018 P =0.0000 T =3.41	0.03 ±0.015 P =0.0000* t =2.48	0.02 ±0.001 P =0.0096* t =3.74	0.02 ±0.003 P = 0.0014* t = 3.40
8	Indirect Bilirubin (mg/dL)	0.16 ±0.012	0.74 ±0.016 P =0.0000 T =56.03	0.16 ±0.012 P =0.0000* t =56.03	0.17 ±0.002 P =0.0000* t =67.98	0.17 ±0.003 P =0.0000* t =67.53
9	AST (IU/L)	35.04 ±0.112	47.01 ±0.428 P =0.0000 T =60.53	36.02 ±0.114 P =0.0000* t =55.47	37.16 ±0.568 P =0.0000* t =30.96	39.04 ±0.127 P =0.0000* t =39.93
10	ALT (IU/L)	27.06 ±0.82	39.12 ±0.613 P=0.0000 T=23.53	28.53 ±0.332 P =0.0000* t =30.92	29.07 ±0.095 P =0.0000* t =32.41	29.36 ±0.288 P =0.0000* t =28.83
11	ALP (IU/L)	65.15 ±0.265	100.07 ±0.42 P =0.0000 T =139.01	65.00 ±0.285 P =0.0000* t =136.50	64.03 ±0.395 P =0.0000* t =123.83	64.01 ±0.488 P =0.0000* t =111.24

The values are expressed as mean of 6 values along with standard deviation and are statistically significant at P <0.05 (*significant), AST - aspartate transaminase, ALT - alanine transaminase, ALP - alkaline phosp

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Discussion

In the present study of course of infection, a gradual increase in the level of parasitaemia was observed as the days progressed from 3 to 7 in the P. berghei infected mice. This is in agreement with the view that parasitaemia increases progressively after inoculation of the infection until the point of death in the absence of suitable treatment (Trampuz et al., 2003; Breman et al., 2001).

In living organisms, liver is an important organ responsible for maintaining and regulating homeostasis. It played important roles in some biochemical pathways which are necessary for growth and to fight against diseases. It also supplies nutrients and energy (Guyton and Hall, 2002). Therefore, maintenance of a healthy liver is essential for the overall well being of an individual.

In the present study, blood glucose was estimated in all the experimental groups. In the P. berghei infected group hypoglycemia was observed with significantly decreased glucose levels. In correlation with the above study, Jaihan et al. (2014) studied the effect of green tea extract on blood glucose levels during P. berghei infection in mice. The low blood glucose levels were observed were observed during malaria infection on day 6. But blood glucose levels were maintained into normal levels in green tea extract treated P. berghei (ANKA) infected mice.

During malaria infection in blood stage, glucose was used to supply the energy and survival of malaria parasite. So increasing of glucose transport across erythrocyte membrane has been described via glucose transporter (GLUT) resulting hypoglycemia in blood stream (Joet et al., 2003). The exact mechanism of hypoglycemia in malaria remains controversial, but insulin appears to play a role in rodent models. It has been reported that hypoglycemia and hyperinsulinemia were only detected when parasitaemia exceeded about 20%, which may explain why hypoglycemia was found in peak parasitaemia, when parasitaemia rose to about 25-30% (Elased et al., 1996; Eltahir et al., 2010). In addition, high levels of tumor necrosis factor (TNF) were responsible for malaria infection and increase glucose uptake by malaria infected erythrocytes (Elased et al., 1996).

Similar study was made by Pattarapo et al. (2017) who reported the alteration of biochemical parameters P. berghei infection in experimental mice. They revealed the hypoglycemia in early infection as indicated by decrease in blood glucose levels. It could be due to the fact that during malaria infection in erythrocytic stage, glucose was rapidly taken up across the parasite membrane through a facilitated hexose transporter (Kraft et al., 2015). This was accompanied with an approximately 100-fold increase in glucose utilization when compared with normal erythrocytes, thus causing hypoglycemia if untreated (Meireles et al., 2017).

In the present study decreased serum total protein level was observed due to reduced concentration of albumin i.e., hypoalbuminemia. Thus decreased albumin and increased globulin content was observed in the P. berghei infected mice at peak level of parasitemia. Our study is in corroboration with the findings of Momoh et al. (2014) who reported significant decrease in plasma total protein in P. berghei (NK 65) infected mice but when treated with Alstonia boonei leaf extract the total protein values significantly increased. Our finding agrees with another report by Uraku (2016) which revealed the decreased serum protein and albumin concentration in P. berghei (NK65) infected Swiss albino mice. But significant elevation in total protein was observed in these mice

treated with leaf extracts of Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera and Cymbopogon citrates.

This may be due to the reduction in protein synthesis. Since malaria parasite causes the destruction of cells that are responsible for protein synthesis. These decreased serum protein concentration in the parasitized untreated group may have resulted from increased protein utilization by the parasite for the building of new protoplasm during multiplication and the host cells for the synthesis of immunoglobulin and acute phase proteins in response to the invading malaria parasites (Al-Omar et al., 2010) or may indicate hepatic malfunction since liver is major source of most serum protein and the synthesis of these proteins are useful indicator of normal hepatic function (Orhue et al., 2005).

Because serum protein are synthesized in the liver, which incidentally is one of the major sites infected by the malaria parasite and any illness such as malaria which cause infection of the liver may lead to fall plasma albumin concentration due to decreased synthesis of albumin. Adeosun et al. (2007) reported that the acute falciparum malaria resulted in significant reduction of total protein albumin and glucose levels in the malarious children.

As well, the total globulin concentration was significantly increased in P. berghei infected mice at peak parasitaemia in our study. The increase in globulin can be attributed to cellular mobilization of T-cells and its complements to mount immunity against parasite activity through synthesis and secretion of antibody molecules that forms part of immunoglobulin proteins. Lunnet et al. (1996) reported a rise in the γ- globulins level at the initial period of the disease which was correlated with the increasing of malaria antibodies. He also reported that stable level hyper gamma-globulinemia is observed in population of endemic malarious areas.

In the present study, serum bilurubin, direct bilurubin and indirect bilurubin have significantly increased in P. berghei infected group at peak parasitaemia on Day 7. This observation with experimental mice agrees with the earlier findings of Igwenyi et al. (2017) who observed increased level of total bilirubin in P. berghei infected mice but treatment with fruit juice extract of Azadirachta indica lowered the level of total bilirubin. Similarly Al-Salahy et al. (2016) reported that patients with falciparum malaria from Hajjah, Northwest Yemen have shown higher levels of bilirubin (total and direct), which is in agreement with our findings.

Ignatius et al. (2008) reported that the liver enzymes leakage and bilirubin, increased with increase in malaria parasite density. Usually, in complicated malaria, raised bilirubin is mainly due to haemolysis of parasitized and non-parasitized RBC and/or hepatocytes damage (Abro et al., 2009). The present study revealed the increase of total bilirubin, direct and indirect bilirubin due to hepatic dysfunction. After artemisinin-based combination therapy (ACT), these bilirubin levels decreased to normal level in ACT treated groups. These values indicate the normal functioning of liver and artemisinin-based combination drugs acted upon to protect the liver against parasitic invasion.

The present study showed that the P. berghei malaria infection in experimental mice elevated the level of enzymes i.e., ALT, AST and ALP when compared to the control group. These findings are in consistent with the previous reports (Gwenyi et al., 2017; Uraku, 2016; Patrick-Iwuanyanwu et al., 2011; George et al., 2011) which revealed the increase in liver enzymes i.e., AST, ALT and ALP during malaria infection. Also, our result is in consist with other studies which reported that majority of

malaria patients have shown elevation in serum activities indicating liver damage (Fabbri et al., 2013).

In mammals, some internal organs and tissues are usually affected by infections and disease conditions but clinical assessment of the extent toxicity is achieved by measuring the activities of certain enzymes that catalyze biochemical reactions in the tissue (Igwenyi et al., 2014) as well as the level of other parameters that may be affected by the infection. This increase in enzyme activities could be attributed to the destruction of the liver parenchyma by the malaria parasite leading to the leakage of the liver enzymes into the blood circulation.

Activities of ALT rises in disease associated with death of hepatocytes like viral hepatitis (George et al., 2011). AST on the other hand is not found to be specific for liver damages but has been found to be a cardiac marker as it is found in cardiac and skeletal muscles (Ujowundu et al., 2011). The serum ALP is related to the function of hepatic cell and increase in serum level of ALP due to increased synthesis of the enzymes in presence of increasing biliary pressure (Emeka and Obidoa, 2009). Generally an increase in this enzyme indicates injury or toxicity to the organ. However, treatment with Artemisinin-based combination drugs caused a significant decrease in the activities of AST, ALT and ALP thereby enzymatic activities in the experimental mice became normal.

Conclusion

According to the present study, different biochemical parameters were altered significantly in P. berghei infection. After treatment with ASAQ, ASSP and AL drugs all the biochemical parameters were restored to normal levels. But among these three artemnisinin-based combination drugs, Artesunate+Amodiaquine (ASAQ) is the most effective drug than ASSP and AL in the present scenario of growing resistance to the antimalarial drugs.

Acknowledgement

The authors are thankful to the Head of the Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur for providing necessary laboratory facilities through UGC-SAP-DRS – Phase III and DST-FIST funding. The first author is also thankful to UGC for granting RGNF to carry out the research work.

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