Chemoprotective Potential of Ethanol Extract of *Ganoderma Lucidum* on Liver and Kidney Parameters in Plasmodium Beghei-Induced Mice

***Adetuyi, B.O and Dairo J.O**
Department of Biochemistry,
College of Natural Sciences,
Joseph Ayo Babalola University,
Ikeji-Arakeji, Ilesa, Osun State.
Nigeria
*krebcycle4u@yahoo.com

Oluwole E.O
Department of Community Health & Primary Health Care,
Lagos State University Teaching Hospital,
Ikeja, Lagos State.
Nigeria

ABSTRACT
The comparative efficacy of ethanolic extract of Ganoderma lucidum in Plasmodium berghei infected mice in comparism with chloroquine, an antimalarial drug of proven efficacy and safety were studied, there is a significant increase ($P<0.05$) in the liver and kidney AST compared to positive control, except for the mice treated with 100 ml of ethanolic extract of Ganoderma lucidum. Whereas there is a significant reduction in the serum AST compared to positive control. The serum ALT shows no statistical significant in the chloroquine extract compared with 100 ml ethanolic extract, but there is a statistically significant increase in 500 ml of ethanolic extract compared with positive control. The liver γGT showed a significant increase when compared with positive control, whereas serum shows a significant reduction in specific activity, when compared with positive control. 100 ml. 250 ml, 500 ml ethanolic extract and chloroquine shows no significant difference when compared with 500 ml, chloroquine and 250 ml ethanolic extract respectively. There is a decrease in body weight of the mice not treated, but a slight increase in the body weight of the mice treated with 100 ml ethanolic extract. But there is no significant difference in the weight of the negative control mice, compared with the mice treated with 500 ml ethanolic extract. There is no significant change in the blood glucose level of the mice treated with 500 ml ethanolic extract compared with the mice treated with 100 ml ethanolic extract, moreover, there is a significant decrease in the body glucose level of the negative control mice compared with the positive control mice, but changes occur as there is an increase in the blood glucose of the mice when treated with the 100 ml and 250 ml ethanolic extracts. Serum ALP shows no statistical significant in all groups except for the negative control mice, which shows an increase when compared with 500 ml ethanolic extract and positive control. Liver shows a significant reduction when compared with positive control except for the mice treated with chloroquine in the liver

KEYWORDS: Ganoderma lucidum, Plasmodiium berghei, chloroquine, ethanolic extract.

INTRODUCTION

Traditional Chinese medicine (TCM) has been practiced in many Asian countries during the past 2 millennia. In TCM, foods play an important role for maintaining and improving health and for preventing and treating disease (Yun 1999). Even with the progress of modern Western medicine in the fields of surgery, radiation therapy, and chemotherapy for cancer treatment, many malignancies can be prevented or treated with proper nutrition.

The general use of dietary supplements in the United States significantly increased during the past 10 years, and the use of dietary supplements and herbal therapies by patients with different diseases like malaria, neurodegeneration and some other notable diseases continues to increase (Go et al., 2001). By tracing the multistep process of carcinogenesis at the cellular levels, it is possible to understand the molecular mechanisms through which the components of foods and botanical dietary supplements affect the development of cancer (Heber et al 1999). Beside that, *Ganoderma lucidum* is reported to have anti-bacterial and antiviral activities (Abbasi et al., 2006), it has been reported to exhibit direct antiviral properties with the following viruses; HSV-1, HSV-2, influenza virus, vesicular stomatitis. Ganoderma mushrooms have been reported to exhibit direct anti-microbial properties with the following organisms; *Aspergillus niger, Bacillus cereus, Candida albicans, and Escherichia coli*.

Ganoderma lucidum (Fr.) Karst. (Ganodermataceae), basidiomycetous fungi, has been used as a medical remedy in China, Korea, and Japan for centuries (Kim 1990). This edible mushroom was considered to preserve the human vitality and promote longevity (Shiao et al 1994). In addition, *Ganoderma lucidum* has been used to treat various human diseases such as allergy, arthritis, bronchitis, gastric ulcer, hyperglycemia, hypertension, chronic hepatitis, hepatopathy, insomnia, nephritis, neurasthenia, scleroderma, inflammation, and cancer (Shiao et al 1994).

Different compounds with various biological activities were extracted from mycelia, the fruiting bodies or spores of *Ganoderma lucidum* and some of them were linked to possible therapeutic effects

MATERIALS AND METHOD

Plant Material: The whole plant of *Ganoderma lucidum* was collected in a village in Edo state. Identification was done in the department of Biological sciences, Joseph Ayo Babalola University.

Extraction: Plant material was air dried under shade and samples were pulverized to a coarse powder to a total of 413g. 200g of the quantity of coarse powder were soaked in 1L ethanol for 12 hours, decanted and concentrated, thus yielding a dark brown extract. This extract was weighed and stored in a refrigerator.

Chemicals: All chemicals were gotten from SIGMA U.S.A.

Malaria Parasite: The chloroquine sensitive Plasmodium beghei (NK 65) was obtained from the National Institute for Medical Research (NIMR), Lagos, Nigeria, and kept in the Animal House Department of Biochemistry, College of Natural Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Ilesa, Osun State, Nigeria. The Parasites were kept alive by continuous re-infestation (I.P) in mice every four days.

Innoculums: Parasitized erythrocytes were obtained from a donor infected mice by cardiac puncture in heparin and made up to 20ml with Normal saline. Animals were inoculated intraperitonially with infected blood suspension (0.2ml) containing 107 parasitized erythrocyte on day zero. Infected mice were parasitaemia of 57% were allocated to six groups of five mice each (Hilou et al., 2006)

Animals and Treatment: 30 Swiss Albino mice (11-25 g BW) obtained from the central animal house, University of Ibadan, Nigeria, were housed in polycarbonate cage in an association of Assessment and Accreditation of Laboratory Animal care-certified animal facility and provided with NIH-07 diet and water *ad libitum*. Animals were allowed to acclamatise for 2 weeks prior to the experiments. Animals were grouped into six (6) with five (5) mice each, and were treated for 14days as follows:
Group A: 1 ml distilled water
Group B: Treated with Plasmodium beghei alone
Group C: Treated with Plasmodium beghei + 500 ml Ethanol extract of *Ganoderma lucidum*
Group D: Treated with Plasmodium beghei + 250 ml Ethanol extract of *Ganoderma lucidum*
Group E: Treated with Plasmodium beghei + 100 ml Ethanol extract of *Ganoderma lucidum*
Group F: Treated with Plasmodium beghei + Chloroquine

RESULT

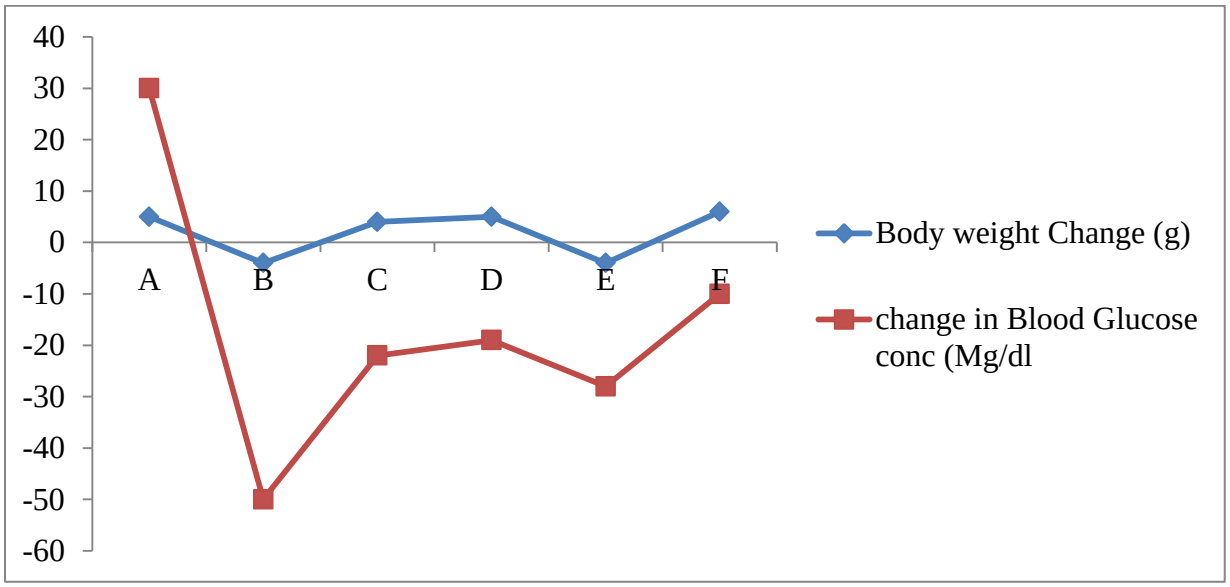

Fig 1: Effect of ethanol extract of *Ganoderma lucidum* on body weight change (g) and blood Glucose concentration in *Plasmodium berghei*-induced mice.

NOTE:
Group A: positive control
Group B: Negative Control
Group C: Treated with *Plasmodium beghei* + 500 ml Ethanol extract of *Ganoderma lucidum*
Group D: Treated with *Plasmodium beghei* + 250 ml Ethanol extract of *Ganoderma lucidum*
Group E: Treated with *Plasmodium beghei* + 100 ml Ethanol extract of *Ganoderma lucidum*
Group F: Treated with *Plasmodium beghei* + Chloroquine

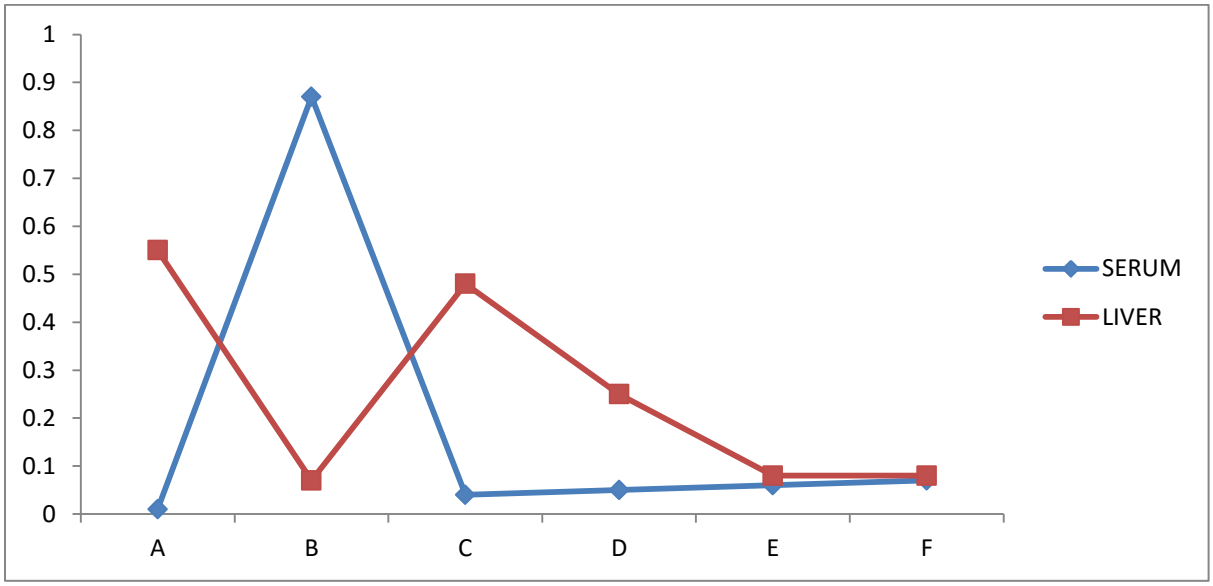

Fig 2: Effect of ethanol extract of *Ganoderma lucidum* on AST Specific activity in *Plasmodium berghei*-induced mice.

NOTE:
Group A: positive control
Group B: Negative Control
Group C: Treated with *Plasmodium beghei* + 500 ml Ethanol extract of *Ganoderma lucidum*
Group D: Treated with *Plasmodium beghei* + 250 ml Ethanol extract of *Ganoderma lucidum*
Group E: Treated with *Plasmodium beghei* + 100 ml Ethanol extract of *Ganoderma lucidum*
Group F: Treated with *Plasmodium beghei* + Chloroquine

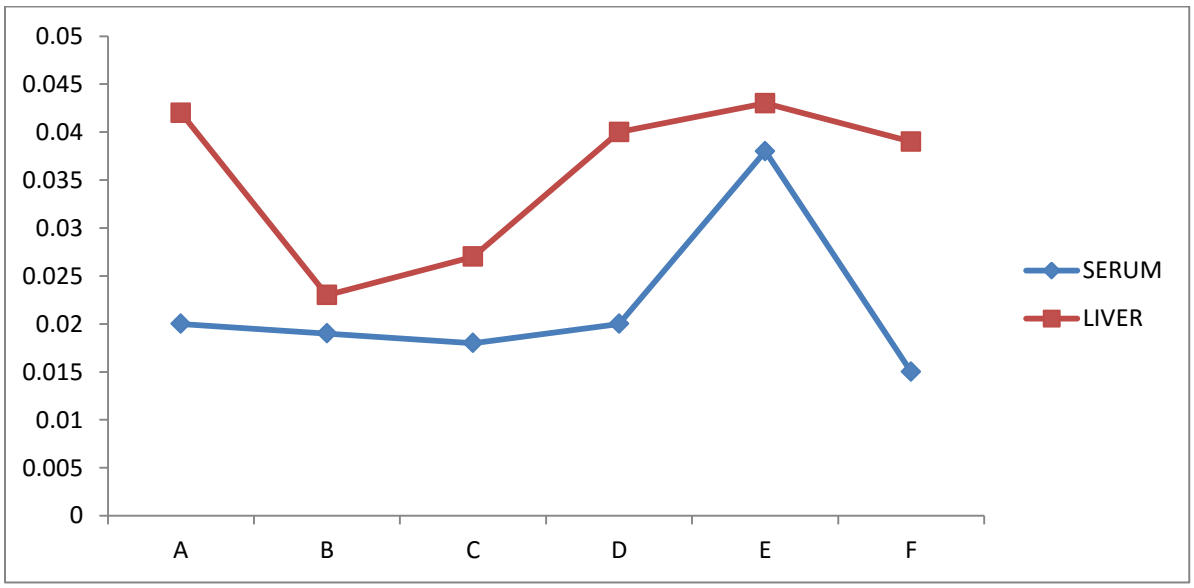

Fig 3: Effect of ethanol extract of *Ganoderma lucidum* on ALT Specific activity in *Plasmodium berghei*-induced mice.

NOTE:
Group A: positive control
Group B: Negative Control
Group C: Treated with *Plasmodium beghei* + 500 ml Ethanol extract of *Ganoderma lucidum*
Group D: Treated with *Plasmodium beghei* + 250 ml Ethanol extract of *Ganoderma lucidum*
Group E: Treated with *Plasmodium beghei* + 100 ml Ethanol extract of *Ganoderma lucidum*
Group F: Treated with *Plasmodium beghei* + Chloroquine

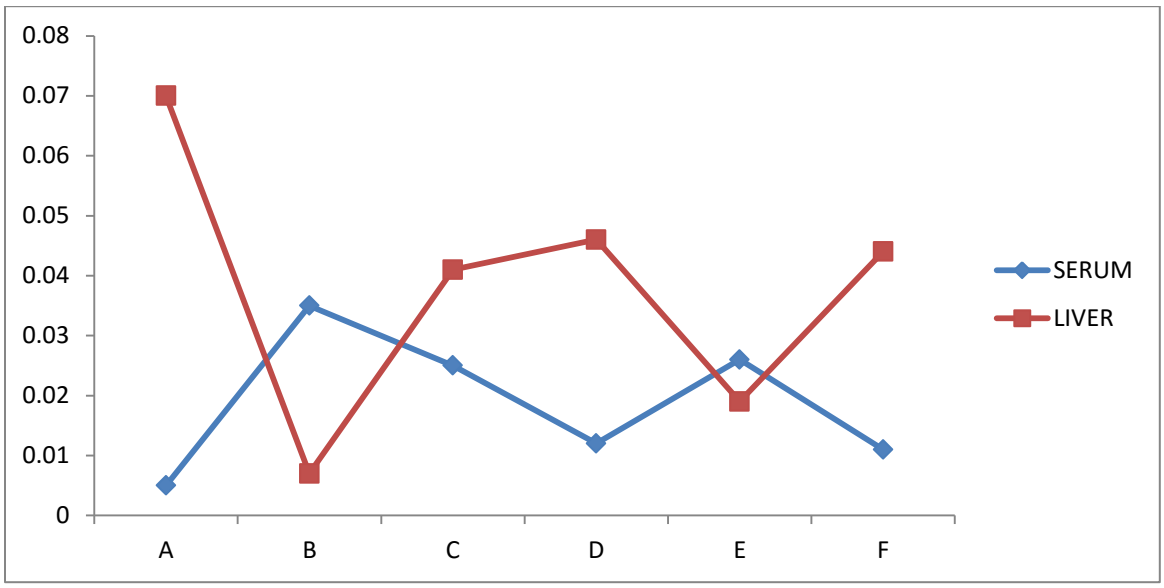

Fig 4: Effect of ethanol extract of *Ganoderma lucidum* on γGT Specific activity in *Plasmodium berghei*-induced mice.

NOTE:
Group A: positive control
Group B: Negative Control
Group C: Treated with *Plasmodium beghei* + 500 ml Ethanol extract of *Ganoderma lucidum*
Group D: Treated with *Plasmodium beghei* + 250 ml Ethanol extract of *Ganoderma lucidum*
Group E: Treated with *Plasmodium beghei* + 100 ml Ethanol extract of *Ganoderma lucidum*
Group F: Treated with *Plasmodium beghei* + Chloroquine

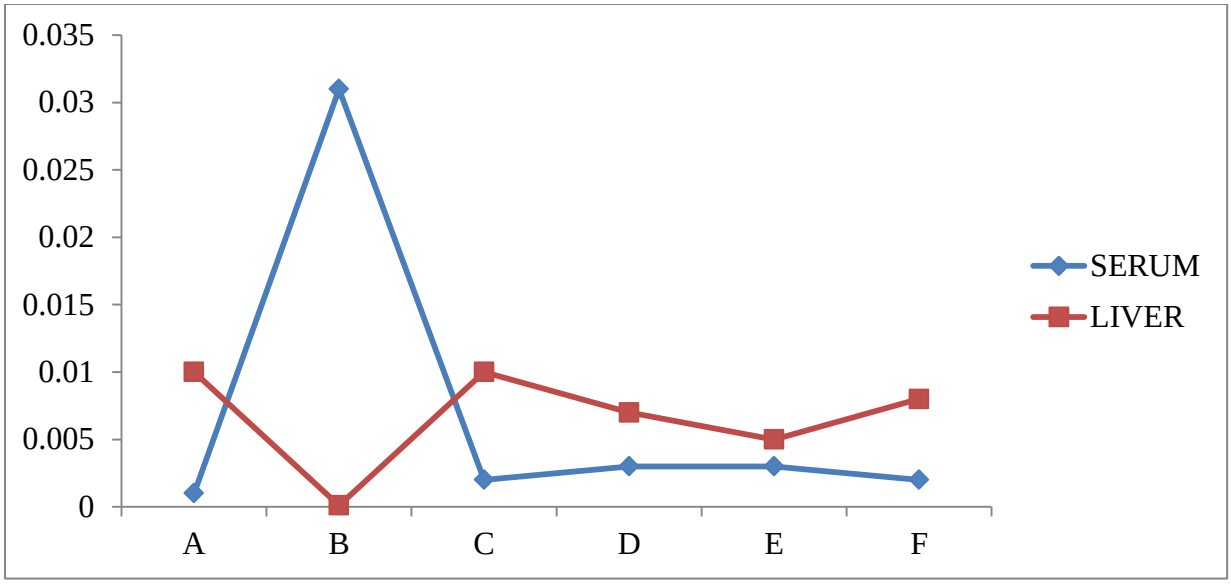

Fig 5: Effect of ethanol extract of *Ganoderma lucidum* on ALP Specific activity in *Plasmodium berghei*-induced mice.

NOTE:
Group A: positive control
Group B: Negative Control
Group C: Treated with *Plasmodium beghei* + 500 ml Ethanol extract of *Ganoderma lucidum*
Group D: Treated with *Plasmodium beghei* + 250 ml Ethanol extract of *Ganoderma lucidum*
Group E: Treated with *Plasmodium beghei* + 100 ml Ethanol extract of *Ganoderma lucidum*
Group F: Treated with *Plasmodium beghei* + Chloroquine

DISCUSSION

When examining the body weight of the mice, there is no significant change in the specific activity of the body weight of mice not treated compared with mice treated with 500ml ethanol extract and there is a significant increase in the body weight of mice ranging from the mice treated with 100ml ethanol extract to the mice treated with chloroquine drug to the mice treated with 250ml ethanol extract, but when comparing it with the positive control, there is a significant decrease in the body weight of mice treated with chloroquine drug.

When examining the blood glucose, there is no significant change in the specific activity of the blood glucose level of the mice treated with 500ml ethanol extract compared with 100ml ethanol extract, and mice treated with 100ml ethanol extract compared with 250ml ethanol extract, whereas, there is a significant increase in blood glucose level of mice treated with chloroquine drug compared with positive control mice.

The measurement of specific activity of various enzymes in the tissues and body fluids play a significant and well known aid in disease investigation and diagnosis (Malomo, 2000) and tissue cellular damage and of course in the assessment of drug or herbal safety. Alkaline phosphate is one of the three enzymes considered in this study for the assessment of safety and effectively of the extract of Ganoderma lucidum. Alkaline phosphate is a marker enzyme for the plasma

membrane and endoplasmic reticulum. Alkaline phosphate is often employed to access the integrity of the plasma membrane (Akanji et al., 1993). The administration of ethanolic extract of Ganoderma lucidum produced a significant reduction in alkaline phosphate compared to the positive control. The reduction in alkaline phosphate in this organ may be adduced to inhibition of enzyme molecule in situ (Umezawa and Hopper, 1982). The reduction in the liver ALP activity following the administration of the plant extract will limit or hinder the adequate transportation of required ions on molecules across the plasma membrane. Researchers have shown that it may also lead to less availability of the phosphate groups for the phosphorylation of ethanolamine and choline needed for the synthesis of major phospholipids, phosphatidylethanolamine and phosphatidylcholine.

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) considered in this study are enzymes which are normally localized within the cell of the liver, heart, gills, muscle, kidney and other organs and are useful marker enzymes in assessing damage in the liver (Chapatwala et al., 1982). Their presence in the serum may give information on the tissue injury or organ dysfunction (Wells et al., 1986), monitoring linkage of liver into the serum has proof to be a very useful tool in the liver toxicity studies.

There is no significant difference in the specific activity of the liver of the mice treated with 500ml ethanolic extract of Ganoderma lucidum, compared with the mice treated with chloroquine drug, but there is a decrease in specific activity of the liver of the mice treated with 100 and 250 ml ethanolic extract compared to the positive control. The decrease in the liver AST may be attributed to the decrease in the functional activity of the liver, resulting in enzyme deactivation. The decreased activity of liver AST is accompanied by increase in serum AST. There is no significant increase in the specific activity of AST in the serum of mice treated with 250 ml ethanolic extract of Ganoderma lucidum, compared with mice treated with 500 ml, but there is an increase in specific activity of the serum of all the mice compared with the positive control. There is no significant difference in the specific activity of the mice treated with chloroquine compared to the positive control mice.

In the ALT assay, there is no significant increase in the specific activity of the serum of the mice treated with 100 ml ethanolic extract, compared with that of the negative control, and the mice treated with 250 ml ethanolic extract, compared to the positive control. Moreover, there is an increase in the serum concentration of the mice treated with 500 ml ethanolic extract compared to the positive control. Also in the liver, there are no significant differences in the specific activity of the mice treated with chloroquine compared with the mice treated with 250 ml ethanolic extract, and the mice treated with 250 ml ethanolic extract, compared with positive control mice, but there is a significant increase in the specific activity of the mice treated with 500 ml of ethanolic extract compared with the positive control mice. In the kidney, there is no significant difference in the specific activity of mice treated with 250 ml of ethanolic extract compared with the mice treated with 500 ml ethanolic extract of the plant material. So also the kidney of the mice treated with 500 ml ethanolic extract shows no significant difference as compared with the mice not treated i.e. negative control mice.

γGT is present in the cell membrane of many tissues, including the kidney, bile duct, pancreas, liver, spleen, heart, brain and seminal vesicle. It involves in the transfer of Amino acid across the cellular membrane and leukotriene metabolism (Goldbergdin, 1980). In γGT assay, there is no

significant differences in the specific activity of the serum of the ice treated with chloroquine drug compared to the mice treated with 250 ml of ethanolic extract of the plant species, and the mice treated with 100 ml ethanolic extract compared with the mice treated with 500 ml ethanolic extract, but there is a significant increase in the serum concentration of all the groups compared to positive control. The liver shows significant decrease in the specific activity of all the mice compared to the positive control, but when comparing the negative control and the mice infected with 500 ml ethanolic extract, there is a significant increase in specific activity.

CONCLUSION

The research work has shown that ethanolic extract of Ganoderma lucidum is efficacious in the treatment of plasmodium berghei in mice

RECOMMENDATION

- There should be a required dosage for the concentration of Ganoderma lucidum that will be used for the treatment of malaria in human body
- Further research should be carried out on this plant materials because it has some other biomedical properties which include cardiovascular disorder, Anticancer, Anti inflammation, immunomodulating effect. e.t.c.
- Patient with an underlying carbohydrate metabolism disorders e.g Diabetes mellitus should reduce the quantity of Ganoderma lucidum in order to prevent worsening of the underlying conditions.

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