



COMPARATIVE STUDY ON THE EFFECTS OF PROPHYLACTIC USE OF CHLOROFORM EXTRACT OF *GANODERMA LUCIDUM*, CHLOROQUINE AND FANSIDAR ON TISSUE CREATININE AND UREA CONCENTRATIONS IN *PLASMODIUM BERGHEI* – INFECTED MICE

A. O. Olusola* and E. E. Olufemi

Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Nigeria.

*Corresponding author email: austinolusola@gmail.com

ABSTRACT

*This research attempts to examine the comparative effects of the prophylactic administration of the chloroform extract of **Ganoderma lucidum** and the conventional anti-malaria drugs – chloroquine & fansidar, on the levels of urea and creatinine in tissues of mice infected with **Plasmodium berghei**. The study involved two control groups (non-parasitized-non treated and parasitized non-treated), and five test groups, namely : mice that received 100 mg, 250 mg and 500 mg of the extract; mice that received 0.1 mg/kg body weight of chloroquine solution and mice that received 0.1 mg/kg body weight of Fansidar suspension. The extract and the drugs were administered for two weeks after which the animals were exposed to malaria infection. Results from this study showed that treatment of the mice with the extract, while showing no significant ($p > 0.05$) difference in creatinine levels in the tissues when compared with both controls, there was a significant ($p > 0.05$) increase in urea levels in liver when compared with the negative control group. From these observations therefore, an inference is drawn that the chloroform extract of **Ganoderma lucidum** contains phytochemicals that inhibit plasmodial activity *in vivo*, which in turn accounts for close-to-normal production of urea in liver of treated mice. Furthermore, the effect of the chloroform extract of **Ganoderma lucidum** and fansidar, appear to be similar in terms of the site of action as demonstrated by the results.*

Key words: *Plasmodium berghei*, *Ganoderma lucidum*, chloroquine, fansidar, urea, creatinine.

INTRODUCTION

Malaria is caused by a parasite called **Plasmodium**, which is transmitted via the bites of infected mosquitoes. In the human body the parasite multiplies in the liver and then infects red blood cells (Greenwood et al., 2005; Olusola, 2014). Symptoms of malaria include fever, headache and vomiting, and usually appear between 10-15 days after the mosquito bite. If not treated, malaria can quickly become life threatening by disrupting the blood supply to vital organs. In many parts of the world, the parasites have developed resistance to a number of antimalarial drugs (Hay et al.,

2004). The key interventions to control malaria include prompt and effective treatment with artemisinin-based combination therapies; use of insecticidal nets by people at risk; and indoor residual spraying with insecticide to control vector mosquitoes (WHO, 2008).

Plasmodium berghei, which was first discovered by Vincke and Lips (1948), infects other mammals other than humans, particularly, rodents in general (Vincke and Lips, 1948). As a result of liver and kidney dysfunction in the later stages of infection by **Plasmodium**, serum creatinine

and urea concentrations are elevated (Ogbadoyi and Tsado, 2009).

Ganoderma lucidum is a polypore mushroom that is soft (when fresh) corky and flat with a conspicuous red-varnished, kidney shaped cap and, depending on specimen age, white to dull brown pores underneath. It lacks gills on its underside and releases its spores through fine pores (David, 1986). It produces a group of triterpenes called ganoderic acids, which have a molecular structure similar to steroid hormones. It also contains other compounds including mannitol and alkaloids (Peterson, 2006). It may possess anti-tumor, immune modulatory and immunotherapeutic activities, supported by studies on polysaccharides, terpenes, and other bioactive compounds isolated from fruiting bodies and mycelia of the fungus (Peterson, 2006). It has been found to inhibit platelet aggregation and to lower blood pressure via inhibition of angiotensin-converting enzyme (Liu and Kurashi, 2006). **Ganoderma lucidum** has recently been demonstrated to possess antiplasmodial effects as a result of isolation of lanostanes and related derivatives which exhibited in-vitro antiplasmodial activity (Bensky and Steven, 2004; Liu and Kurashi, 2006; Moradali and Mostafavi, 2006; Peterson, 2006; Adams et al., 2010).

Chloroquine is a 4-aminoquinoline drug used in the treatment or prevention of malaria. Inside red blood cells, the malaria parasite must degrade hemoglobin to acquire essential amino acids which the parasite requires for its protein synthesis and energy metabolism. Digestion is carried out in the vacuole of the parasite cell. During this process, the parasite produces toxic and soluble heme molecule. The heme moiety consists of a porphyrin ring called Fe (II) – protoporphyrin IX (FP). To avoid destruction by this molecule, the parasite bio-crystallizes heme to form hemozoin, a non – toxic molecule. Hemozoin collects in the digestive vacuole as insoluble crystals. Chloroquine enters the red blood cell, inhabiting parasite cell and digestive

vacuole by simple diffusion chloroquine then becomes protonated (to CQ^{2+}), as the digestive vacuole is known to be acidic (pH 4.7). The drug caps hemozoin molecules to prevent further biocrystallization of heme, thus leading to hemozoin build-up. Chloroquine binds to heme (or FP) to form what is known as the FP- chloroquine complex; this complex is highly toxic to the cell and disrupts membrane function. Action of the toxic FP- chloroquine and FP results in cell lysis and ultimately, parasite cell auto digestion. In essence, the parasite cell drowns in its own metabolic products (Hempelmann, 2007).

Fansidar® is a combination anti-malarial drug containing the sulphonamide antibacterial sulfadoxine and the anti-parasitic pyrimethamine. Both sulfadoxine and pyrimethamine are antifolates; they inhibit the production of enzymes involved in the synthesis of folic acid which is required by the parasites for growth (Chulay et al., 1984).

In this study, the effect of the chloroform extract of **Ganoderma lucidum** in comparison with those of the conventional drugs, namely chloroquine and fansidar®, on the levels of creatinine and urea in tissues of mice infected with **Plasmodium berghei** was evaluated.

MATERIALS AND METHODS

Parasite

Plasmodium berghei (NK-65) procured from Nigerian institute of Medical Research, Yaba, Lagos (Nigeria) was employed in the present study.

Experimental design

Forty nine (49) Swiss albino mice weights 15-22 g obtained from central animal house of University of Ibadan, Nigeria were used. The mice were fed with pelletized growers mash and water *ad libitum*.

The animals were divided into seven groups of 7 mice per group as follows:

Group I: normal control (N-CTR); the mice were administered normal saline intraperitoneally.

Group II: infected control (I-CTR); the mice were inoculated with 10^6 parasitized erythrocytes intraperitoneally but not treated.

Group III CHL₁₀₀: mice in this group received 100 mg of the chloroform extract of **Ganoderma lucidum** per kg body weight per day prior to inoculation.

Group IV CHL₂₅₀: mice in this group received 250 mg of the chloroform extract of **Ganoderma lucidum** per body weight per day, prior to inoculation.

Group V CHL₅₀₀: the mice in this group received 500 mg of the chloroform extract of **Ganoderma lucidum** per body weight per day, prior to inoculation.

Group VI: the mice in this group were administered a solution (0.1 mg/kg body weight) of chloroquine per day prior to inoculation.

Group VII: mice in this group were given a solution (0.1 mg/kg body weight) of fansidar per day, prior to inoculation. Administration of the extract and drug solutions to mice was done orally via gavage for 14 days.

Collection of samples

The animals were sacrificed by stunning and blood samples were collected by jugular puncture into properly labeled anticoagulant-free bottles. Sera samples were obtained by spinning at 3,500 rpm for about 15 minutes and kept at 4°C until required. The liver and kidneys of the animals were removed and stored in 0.1 mM phosphate buffer at pH 6.5. These tissues were homogenized in normal saline solution with mortar and pestle and then centrifuged. The supernatants obtained were stored at 4°C till required.

Plant Material

Fruiting bodies of **Ganoderma lucidum** were obtained locally from an open forest at Ipele in Owo Local Government Area of Ondo State, Nigeria. The plant material was identified by a taxonomist in the department of plant science, University of Benin, Benin City, Nigeria, where voucher samples were kept for reference.

Preparation of plant extract

Fruiting bodies of **Ganoderma lucidum** were sorted, washed to remove debris and dust particles and then dried under shade for two weeks. The dried materials were cut into pieces and grounded into powder using mechanical grinder. One hundred (100) grams of the powdered extract was soaked in 1000 ml chloroform at room temperature for 72 hours after which the resulting mixture was filtered using Whatman (number 54) filter paper. The resulting filtrate was air-dried at room temperature in Petri dishes until no smell of the solvent was noticed. The residue obtained was weighed and kept in an air-tight container at 4 °C. Percentage yield was determined from the weight of the dried residue.

Biochemical analyses

Creatinine and urea levels in the serum and tissues were estimated using the procedure described in the user manuals of the creatinine and urea kits obtained from Randox Laboratories, London.

RESULTS

Table 1 shows the effects of chloroform extract of **Ganoderma lucidum**, chloroquine and fansidar® on the levels of creatinine in the serum, liver and kidneys of mice infected with **Plasmodium berghei**. The levels of creatinine were significantly ($P < 0.05$) higher in the liver and kidneys of non-infected mice (positive control) when compared to infected, untreated mice (negative control) creatinine levels in liver in all the groups were not significantly different ($P > 0.05$) from one another.

Treatment of mice with varying doses

of chloroform extract of **Ganoderma lucidum** significantly ($P>0.05$) increased creatinine levels in serum and kidney when compared with negative control. These groups which received the chloroform extract also showed a significant reduction ($P>0.05$) in kidney creatinine levels and a non-significant ($P>0.05$) difference in serum creatinine levels when compared with positive control.

Treatment of mice with 100 mg/kg of the extract showed a significant ($P>0.05$) reduction in serum creatinine levels with a corresponding significant ($P>0.05$) increase in kidney creatinine levels when compared to mice that received 250 mg/kg and 500 mg/kg of the extract.

The group which received chloroquine treatment showed significant ($P>0.05$) increase in serum and kidney creatinine

levels when compared to negative control. The group also reflected a significant ($P>0.05$) reduction in creatinine levels in serum, but a non-significant ($P>0.05$) reduction in kidney creatinine levels, when these are compared to positive control.

The group which received fansidar® treatment showed no significant ($P>0.05$) difference in creatinine levels in serum but showed a significant ($P<0.05$) increase in creatinine levels in the kidney when compared to the infected but not treated group. The group also showed a significant ($P<0.05$) reduction in serum creatinine levels and no significant reduction in kidney creatinine levels when compared with normal control.

Table 1: Effects of prophylactic administration of chloroform extract of *G. lucidum*, chloroquine and fansidar on creatinine levels of serum, liver and kidney of mice infected with *Plasmodium berghei*.

	GROUPS	Serum creatinine ($\mu\text{mol/L}$) $\times 10^{-3}$	Liver creatinine ($\mu\text{mol/mg protein}$) $\times 10^{-3}$	Kidney creatinine ($\mu\text{mol/mg protein}$) $\times 10^{-3}$
1	N-CTR	16.97 \pm 1.68 ^{d,e,f}	25.92 \pm 4.80 ^a	55.61 \pm 6.42 ^{f,g}
2	Infected-CTR	7.07 \pm 1.12 ^a	16.50 \pm 4.26 ^a	5.66 \pm 0.94 ^a
3	CHL ₁₀₀	15.55 \pm 0.61 ^{c,d,e}	20.74 \pm 4.55 ^a	34.88 \pm 3.13 ^e
4	CHL ₂₅₀	19.79 \pm 1.02 ^f	14.14 \pm 1.19 ^a	22.15 \pm 2.67 ^{c,d}
5	CHL ₅₀₀	20.74 \pm 1.39 ^f	24.51 \pm 1.59 ^a	13.20 \pm 1.25 ^{a,b,c}
6	CQ	12.73 \pm 1.83 ^{b,c}	17.44 \pm 5.42 ^a	51.37 \pm 2.87 ^f
7	SP	6.60 \pm 0.72 ^a	13.20 \pm 1.91 ^a	49.96 \pm 5.11 ^f

The results are presented as means \pm SEM of seven (7) determinations. Statistical comparison is strictly within the same tissue. Values with the same superscripts do not differ significantly while values with different superscripts are significantly different from one another.

The effects of chloroform extract of **Ganoderma lucidum** Chloroquine (CQ) and Fansidar® (SP) on the levels of urea in serum liver and kidney of mice infected

with **Plasmodium berghei** is presented in Table 2. The levels of urea in liver were significantly ($P<0.05$) higher in the normal control group than that obtained in the infected group, and there was no significant ($P>0.05$) difference in the kidney urea levels of both groups. Serum urea concentrations of all groups were not significantly ($P>0.05$) different from one another. Treatment of mice with

Comparative study on the effects of prophylactic use of chloroform extract of ganoderma lucidum, chloroquine and fansidar on tissue creatinine and urea concentrations in plasmodium berghei – infected mice

chloroform extract of **G. lucidum** did not show significant ($P>0.05$) difference in liver urea levels when compared with both infected and normal controls. However, group 4 (which received 250 mg of the extract) showed a significant reduction in kidney urea levels when compared with both normal and infected controls; while groups 3 and 5 (CHL₁₀₀ and CHL₅₀₀) showed a non-significant ($P>0.05$) reduction in kidney urea levels relative to both normal and infected controls.

Treatment of mice with graded doses of the extract did not show significant ($P>0.05$) difference in serum, liver and

kidney urea concentrations. The group that received chloroquine (CQ) treatment showed significant ($P<0.05$) reduction in both liver and kidney urea levels when compared with normal control.

Mice treated with fansidar also showed a significantly ($P<0.05$) increased serum urea concentrations with respect to normal control, but showed significant ($P<0.05$) reduction in the liver and kidney urea levels when compared to both controls.

Table 2: Effects of prophylactic administration of Ganoderma lucidum, chloroquine and fansidar® on levels of urea in serum, kidney and liver infected with Plasmodium berghei.

	GROUPS	Serum Urea mmol/L	Liver Urea mmol/mg protein	Kidney Urea mmol/mg protein
1	N-CTR	4.98±0.54 ^a	68.84± 6.28 ^d	126.80±4.39 ^e
2	Infected-CTR	7.71 ± 1.60 ^b	49.67±5.39 ^{b,c}	116.09±9.89 ^e
3	CHL 100	6.00±0.70 ^{a,b}	59.49±5.45 ^{c,d}	103.19±5.59 ^{c,d,e}
4	CHL 250	6.14±1.06 ^{a,b}	51.82±5.72 ^{b,c,d}	88.66±5.17 ^{b,c,d}
5	CHL 500	6.34±1.09 ^{a,b}	57.85±7.01 ^{b,c,d}	103.74±3.49 ^{c,d,e}
6	CQ	7.85± 0.88 ^b	49.74 ±4.67 ^{b,c}	88.15±6.59 ^{b,c,d}
7	SP	6.82 ±0.36 ^b	53.25±8.63 ^{b,c,d}	101.96±5.60 ^{c,d,e}

The results are presented as means ± SEM of seven (7) determinations. Statistical comparison is strictly within the same tissue. Values with the same superscript do not differ significantly while values with different superscripts are significantly different from one another.

DISCUSSION

The serum and kidney levels of creatinine in untreated mice were significantly higher than those of uninfected mice in this study. This may be due to the fact that there was a continued reduction in the weights of the infected mice due to tissue protein degradation as

the infection progressed. Also, treatment of mice with the chloroform extract of **G. lucidum** showed significant increases in serum and kidney levels of creatinine when compared to untreated mice. The possible explanation for these observations is that, with respect to normal control, the creatinine levels of these mice are unaffected by chloroform extract of **G. lucidum**. Since creatinine is excreted at a rate proportionate to muscle mass, these levels of creatinine observed in kidney and

serum of mice treated with the extract, showed that the kidneys were not adversely affected.

Treatment of mice with chloroquine and fansidar® also showed similar results to those treated with the extract and this further lends credence to the fact that their creatinine levels were not significantly affected and therefore their kidneys were functioning relatively optimally.

This research also showed that urea levels in liver of untreated mice were significantly lower than those of uninfected mice, while urea levels in serum and kidney of both groups were not significantly different from each other. The possible explanation for what is observed is that the species of plasmodium involved (**Plasmodium berghei**) has little adverse effect on the kidneys of the mice involved. This is justified by the relative constancy observed in the levels of urea recorded in both normal and infected controls. However, with the marked reduction in urea levels in liver, it is therefore evident that **Plasmodium berghei**, in the sporozoite stage of its life cycle, like other **Plasmodium** species, plays a role in reduction of urea production by the liver thereby altering the liver function. This suggests an early stage of liver dysfunction and it agrees with earlier reports (Ogbadoyi and Tsado, 2009).

Treatment of mice with varying doses of the extract showed significant increases in liver urea concentration when compared to that of infected-untreated mice, while there was significant difference in kidney urea levels when compared to both controls. Prophylactic treatment of mice with fansidar also showed similar trend. Furthermore, treatment with chloroquine showed similar results with that of untreated mice. This explains that there are phytochemicals in the chloroform extract of **Ganoderma lucidum** which significantly inhibited hepatic plasmodial activity. This

observation is in agreement with earlier findings (Adams et al., 2010), which established the presence of lanostanes in the plant extract and showed their ability to inhibit plasmodial activity **in vitro**. Comparing the activity of the chloroform extract of **Ganoderma lucidum** with that of chloroquine, the relative differences in liver urea levels can be explained in terms of their sites of action. It is evident that chloroquine exhibits a lesser effect on the liver when compared with the chloroform extract. The results also suggest that the most likely site of action of the chloroform extract of **Ganoderma lucidum** is the hepatocytes and less likely to be erythrocytes. This contrasts with that of chloroquine, which is already known to inhibit heme biocrystallization in erythrocytes. (Hempelmann, 2007). The effect of the chloroform extract of **Ganoderma lucidum** and the drug fansidar, appear to be similar in terms of the site of action as demonstrated by the results.

SUMMARY AND CONCLUSION

The study presents a report on the effect of chloroform extract of **Ganoderma lucidum**, chloroquine and fansidar on the urea and creatinine levels of mice infected with **Plasmodium berghei**. There have been reports of the antiplasmodial activity of **Ganoderma lucidum in vitro** (Adams et al, 2010), but this study gives an evidence of such investigation in mice whose physiology is comparable and predictive to that of humans. This study also gives credence to the claim that **Plasmodium berghei** is capable of causing damage to essential organs such as the liver, just like most other species of **Plasmodium** (Janse et al., 2006). It is of interest that the results presented here suggest that the chloroform extract of **Ganoderma lucidum** exerts

Comparative study on the effects of prophylactic use of chloroform extract of ganoderma lucidum, chloroquine and fansidar on tissue creatinine and urea concentrations in plasmodium berghei – infected mice

significant effect on the liver of mice.

REFERENCES

- Adams, M., Christen, M., Plitzko, I., Zimmermann, S., Brun, R., Kaiser, M. and Hamburger, M. (2010). Antiplasmodial lanostanes from the **Ganoderma lucidum** mushroom J.Nat. Prod. 73(5): 897-900.
- Bensky, D., and Steven C., (2004) Chinese Herbal Medicine, material medica, third edition. Brosci. Brotechnol. Biochem., 68(4): 881-887.
- Chulay, J.D., Watkins, W.M., and Sixsmith, D.G. (1984) Synergistic antimalarial activity of pyrimethamine and sulfadoxine against Plasmodium falciparum in vitro. Am J Trop Med Hyg 33: 325–330.
- Greenwood, B.M., Bojang, K., Whitty, C.J., and Targett, G.A. (2005). “Malaria” Lancet 365 (9469): 1487-98.
- Hay, S., Guerra, C., Tatem A., Noor, A. and Snow, R. (2004) “The global distribution and population at risk of malaria: Past, present and future”.
- Hempelmann, E. (2007) Hemozoin biocrystallization in **Plasmodium falciparum** and the antimalarial activity of crystallization inhibitors. Parasitol Research 100(4):671-676.
- Janse, C.J., Ramesar, J. and Waters, A. (2006). “High-efficiency transfection and drug selection of genetically transformed blood stages of the rodent malaria parasite **Plasmodium berghei**. Nature protocols 200n, 1:346-356.
- Liu, J. and Kurashi, K. (2006) “Structure activity relationship for inhibition of 5 alpha reductase by triterpenoids isolated from **Ganoderma lucidum**” Bioorganic Med. Chem 14(24): 8654-60.
- Moradali, M.F. and Mostafavi, H. (2006). “Investigation of potential antibacterial properties of methanol extract from fungus ganoderma applanatum chemotherapy 52 (5): 241-4.
- Ogbadoyi, E.O and Tsado, R.D. (2009) Renal and Hepatic dysfunction in malaria, patients in Minna, North central Nigeria. African Journal of infectious diseases, 2007, 1(1): 57-64.
- Olusola, A.O. (2014)**. Effects of **Plasmodium falciparum** on the antioxidant status of Nigerian children. Proceeding of the 4th Int. Conference on Humanities, Science and Sustainable Development held April, 8-9, 2014 at the University of Benin, Benin City. Pp167-170.
- Peterson R.R, (2006). “Ganoderma – a therapeutic fungal biofactory. Phytochemistry 67(18): 1985-2001.
- Vinke, T.H and Lips, M. (1948) Un nouveau, plasmodium d'un rongeur sauvage du Congo. Plasmodium berghei N.sp Annales de la societe Belge de Medecine Tropical 28, 97-