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Changes in Lipid Profile in *Plasmodium berghei* Anka 65 Infected Mice Treated with Ethanolic Extracts of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citratus*

Uraku Anayo Joseph, Onuoha Stanley Chukwudozie*

Department of Biochemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

Email address
sconuoha@yahoo.com (O. S. Chukwudozie)

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Abstract
Changes in lipid profile in *Plasmodium berghei* Anka 65 infected mice treated with ethanolic extracts of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera and Cymbopogon citratus*. Eighty four (84) swizz mice of both sexes were used. All the mice were infected intraperitoneally with 0.2 ml parasitized blood suspension and parasitemia assessed by thin blood films stained with Geimsa stain after seventy two hours. The mice were divided into 6 groups namely; A, B, C, D, E and F. Groups B, C, D and E were subdivided into three (3): B1, B2, B3, C1, C2, C3, D1, D2, D3, E1, E2 and E3. Both groups and subgroups contained 6 mice each. The subgroups were treated with the extracts of *Spilanthes uliginosa (Sw), Ocimum basilicum, Hyptis spiligera* and *Cymbopogon citratus* each for five (5) consecutive days with 200, 400 and 800 mg/kg body weight via oral intubation daily respectively. The results showed dose dependent significant ($P<0.05$) reductions in the plasma level of total cholesterol, triacylglycerol and low density lipoprotein cholesterol (LDL-C) and dose dependent significant ($P<0.05$) increase in high density lipoprotein cholesterol (HDL-C) of the parasitized treated mice. Overall, the dose dependent effects were in the order of: 5 mg/kg body weight of chloroquine > 800 mg/kg > 400 mg/kg > 200 mg/kg body weight of the plant extracts with the efficacy of the plants in the order of: *H. Spicigera* > *O. basilicum* > *C. citrates* > *S.uliginosa* (Sw) with minor variations.

1. Introduction

Malaria has been described as a disease of the poverty and underdevelopment, is considered the most complex and overwhelming health challenge facing humanity in the vast majority of the developing tropical and subtropical countries, with over 300 to 500 million cases and 2 to 3million deaths per year (WHO, 2000). *Plasmodium falciparum* is the most dangerous and pathogenic among the four species causing majority of infections in human and account for an estimated 1.4 and 2.6 million deaths per year in this region (WHO, 1997, 2003). When the parasites invade the human blood, erythrocytes become infected following attachment and invasion by merozoite. Various phases of parasite development include trophozoites and schizonts. In vitro, the parasite grows in 5-10% human serum in an atmosphere of low oxygen (WHO, 1997).

Transitory changes in the plasma levels of lipids such as cholesterol and triglycerol have been observed many years ago by many authors in different acute infections (Das *et al.,* 1996). Hypocholesterolemia, hypertriglyceridemia and extreme decrease in HDL and LDL fractions were observed in complicated and uncomplicated malaria infections (Devendra *et al.,* 2005). The magnitude of these changes seems related to the severity of malaria (Krishna *et al.,* 2009). Hyperlipidemia, a hallmark of malarial infection which may result in depletion of natural antioxidants and facilitates the production of reactive oxygen species (ROS) which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, DNA and exert cytotoxic effects on cellular components have been documented (Al-omar *et al*., 2010). Thus, increased ROS and impaired antioxidant defense contributes to initiation and progression of micro and macro vascular complications in malaria (Kittl *et al*., 1992).

Numerous attempts has been made to control the disease by using vector control measures and/or chemoprophylaxis but they have had limited success (Trigg & Kondrachine, 1998). The use of classical drugs of chloroquine and primaquine has been frustrated by the resistance of malarial parasites to these drugs (Olorunniyi & Morenikeji, 2014). There is therefore a need to search for drugs with novel modes of action to treat the disease.

Plants which serve as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health. Since ancient times medicinal plants have continued to be an important therapeutic aid for alleviating the ailments of humankind. Therefore, to search for antimalarial drug from plant origin cannot be neglected, more especially, that the antimalarial drugs in use today (quinine and artmisinnin) were isolated from plants (Olorunniyi & Morenikeji, 2014).

Locally, *Spilanthes uliginosa (Sw), Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citratus* have been used trado-medically for various medicinal purposes. *Spilanthes uliginosa (Sw),* commonly known as ground pepper is an annual herb or short - lived perennial approximately half meter tall (Shanthi and Amudha, 2010). It belongs to the family compositae, tribe *Helianthae* and the sub - tribe E*cliptinae.* About sixty species of *Spilanthes* have been reported from various parts of the world including India, and they have characteristic flower heads which distinguishes individual species. It originated in Africa and South American tropics but is now distributed in tropics and sub-tropics of the world (Kadir *et al*., 1989). The genus occurs widely in damp pastures, at swamp margins, on rocks near the sea and as a weed of road - sites and cultivations (Ramsewak *et al.,* 1999). The plant is traditionally used in Asia, Central and South America and in Africa as therapeutic diets (Tawer *et al*., 2010) The leaves and part thereof are used by indigenous herbalists in the treatment of toothache, headache, convulsion/epilepsy, malaria, thyphoid fever, tuberculosis, snake bites and in wound healing among others (Shanthi and Amudha, 2010).

Basil (*Ocimum basilicum*) is an aromatic, low growing herb. The plants have a bright green to purple ovate colour leafs, and are grown in warm, tropical climate. It belongs to the botanical family of *Ocimum basilicum*, which is commonly known as mint. The basil leaves are known to have many medicinal and other healing properties (Gabi *et al.,* 2012). Studies have shown that basil plant extracts and oils can serve as anti-anxiety and anti-depressant due to its ethanolic composition (Edris and Farrag, 2003). Essential oil of basil, obtained from its leaves, has demonstrated the ability to inhibit the growth of *Staphylococcus*, *Enterococcus, Shigella* and *Pseudomonas*, (Elgayyar *et al.,* 2001). The eugenol is reported as an important component of basil's volatile oils and can block the activity of *Cyclooxygenase* (COX) and hence acts as an anti-inflammatory agent. The oil helps in the relief of rheumatoid arthritis and inflammatory bowel conditions (Suppakul *et al.,* 2003) among others.

Hyptis spicigera is species of scent leaf, widely distributed in tropical and warm temperate regions. About 50 species of the plant are found in Nigeria (Coker *et al.,* 2000). It is used in treatment of various kinds of ailment such as upper respiratory tract infections, diarrhea, headache, pneumonia, fever and cholera (Gabi *et al.,* 2012)

Cymbopogon citratus commonly known as lemongrass is a tropical perennial herb belonging to the family Poaceae (true grasses). It is commonly used in traditional Indian, Chinese, and Brazilian medicines (Negrelle and Gomes, 2007). *Cymbopogon citratus* has been shown to be effective in the treatment of fever and infections, headaches, stomach aches, and rheumatic pain (Agbafor and Akubugwo, 2007). It is also reported to act as sedative, antispasmodic, analgesic, anti-inflammatory, and antihypertensive agents (Vanisha and Hema, 2012). However, the scientific evidence for its alleged therapeutic efficacy is still lacking.

In this study the lipid profile in *Plasmodium berghei* Anka 65 infected mice treated with ethanolic extracts of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citratus* was investigated

2. Materials and Methods

2.1. Collection of Plant Material

Fresh leaves of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citratus* were collected from Ogboji - Agoutu Ezzagu in Inyaba Development Centre of Ebonyi State, Nigeria. The plants were identified and authenticated by a botanist from the Department of Applied Biology, Ebonyi State University, Abakaliki were voucher specimen were deposited. The leaves of the plant were cleaned, air-dried inside a room and then pounded into fine powder using an electric blender. The powder was stored in an airtight container and kept in a cool, dry place.

2.2. Extraction of Plant Materials

Exactly 150 g of powder samples of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera* and *Cymbopogon*

citratus were soaked in 500 ml of ethanol each for 24 hours. They were filtered into a graduated beaker and exposed to mild heat at 40°C in water bath until a semi solid extracts were obtained.

2.3. Experimental Animals

Thirty Swizz albino mice aged 2 months weighing 17-34 g of both sexes were obtained from animal house of Nnamdi Azikiwe University Awka, Anambra State and transferred to Animal house Department of Biochemistry, Ebonyi State University, Abakalki. The animals were housed in metal cages under controlled conditions and acclimatized for 7 days under standard environment conditions and fed *ad-libitum* on their normal diets.

2.3.1. Rodent Parasite (*Plasmodium berghei Anka 65*)

The rodent parasite was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria and maintained alive in mice by continuous intraperitoneal passage in mice after every 5 days. The re-infected mice were moved to the Animal House of Department of Biochemistry, Faculty of Biological Science, Ebonyi State University Abakaliki where the study was carried out. Prior to the start of the study, one of the infected mice was kept and observed to reproduce signs of diseases similar to human malarial infection.

2.3.2. Inoculation of Animals

The mice were infected with parasites consisting of 1×10^7 of *P. berghei* parasitized erythrocytes per ml. This was carried out by determining both the percentage parasitaemia and erythrocytes count of the donor mouse and diluting the blood with phosphate buffer saline pH 7.4 in proportions indicated by both determinations. Each mouse was inoculated intraperitoneally with 0.2 ml of infected blood containing 1×10^7 *P. berghei* parasitized red blood cells. Parasitaemia was assessed by thin blood film made by collecting blood from the cut tip of the tail and this was stained with Geimisia stain (WHO, 2000)

2.4. Experimental Design

Eighty four (84) swizz mice of both sexes were used. All the mice were injected intraperitoneally with standard inoculums of 1×10^7 *P. berghei* Anka 65 infected erythrocytes on the first day. Seventy two hours later, the mice were divided into 5 groups namely; A, B, C, D, E and F of 6 mice each. Groups B, C, D and E were subdivided into three (3): B_1, B_2, B_3, C_1, C_2, C_3, D_1, D_2, D_3, E_1, E_2 and E_3. The subgroups were treated with the extracts of *Spilanthes uliginosa (Sw), Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citratus* each for five (5) consecutive days with 200, 400 and 800 mg/kg body weight via oral intubation daily respectively. Two control groups, A and F were used. The negative control (A) was treated daily with 5 ml/kg normal saline while positive control group (F) was treated with 5 mg/kg body weight of chloroquine. All groups were given water and fed *ad libitum*. On the sixth day mice were starved overnight, sacrificed and liver was collected for various biochemical estimations.

2.5. Preparation of Serum

Fasting blood was collected from each mouse into a sterile, plain tube, and then it was centrifuged at 1,200 × g for 5 min at room temperature to obtain the serum sample, which was stored frozen at -20°C until analyzed.

2.6. Lipid Profile Studies

2.6.1. Determination of Triglyceride (Quinica, 1978)

Test tubes were labeled blank, standard and sample. 0.01ml of standard solution and sample were pipetted into the test tube labeled standard and sample respectively. Then, 0.01 ml of distilled water was taken to blank test tube. Zero point zero one millilitre of triglyceride working reagent (40 mM of pipes buffer pH 7.5 + 6 mM of 4 - chlorophenol + 4 - aminoantipyrine + 1 mM of APT + 5 Mm of $mgCl_2$ + 40 quinoneimine + HCL + $4H_2O$ µg of glycerol lipase + 155 µg of glycerol - 3 - phosphate oxidase + 500 µ/l of lipase) was added to all the test tubes and mixed thoroughly. The test tubes were incubated at 37°C for 5 minutes. The absorbance of the sample and standard was read in a visible spectrophotometer against the reagent blank within 60 minutes at 500 nm using the formular.

$$\text{Triglyceride concentration (mg/dl)} = \frac{\text{Change in absorbance of the sample x Concentration of the standard}}{\text{Change in absorbance of the standard x 1}}$$

2.6.2. Determination of Total Cholesterol (Allian and Rosechlaw, 1979)

Test tubes were labeled blank, standard and sample. Standard solution (0.01 ml) and sample were pipetted into the test tube labeled standard and sample respectively. Then, 0.01 ml of distilled water was taken to blank test tube. 0.0ml of Cholesterol working reagent (40 mM of pipes buffer P^H 7.5 + 6 mM of 4 – chlorophenol + 4 – aminoantipyrine + 1 mM of APT + 5 Mm of $MgCl_2$ + 40 quinoneimine + HCL + $4H_2O$ µg of glycerol lipase + 155 µg of glucerol - 3 - phosphate oxidase + 500 µ/l of lipase) was added to all the test tubes and mixed thoroughly. The test tubes were incubated at 37°C for 5 minutes. The absorbance of the sample and standard was read in a visible spectrophotometer against the reagent blank within 60 minutes at 500 nm and total cholesterol was determined using the formular.

$$\text{Triglyceride concentration (mg/dl)} = \frac{\text{Change in absorbance of the sample x Concentration of the standard}}{\text{Change in absorbance of the standard x 1}}$$

2.6.3. Determination of High Density Lipoproteins (HDLS)

Test tubes were labeled blank, standard and sample. Zero point two millilitres of serum and standard solution were pepittted into the test tube labeled sample and standard respectively. Zero point five milliliters (0.5 ml) of HDL - Cholesterol working reagent was also added to the tubes. They were properly mixed and incubated for 10 minutes at 25^{o}C followed by centrifugation for 10 minutes at 4,000 rpm. Thereafter, the supernatant was used to determine the concentration of HDL - Cholesterol. Another set of test tubes were selected and labeled standard, sample and blank. Zero point one millilitre of distilled water, supernatant of sample, supernatant of standard solution were added to the tubes labeled blank, sample and standard respectively. Zero point one millilitre of HDL - cholesterol working reagent was also added to all the test tubes and mixed thoroughly. They were incubated at 25^{o}C for 10 minutes and the absorbance read in a visible spectrophotometer within 60 minutes at 500 nm against blank using the formula.

$$\text{HDL} - \text{Cholesterol concentration (mg/dl)} = \frac{\text{Change in absorbance of the sample x Concentration of the standard}}{\text{Change in absorbance of the standard x 1}}$$

2.6.4. Determination of Low Density Lipoprotein (LDLS)

The equation method of Friedewald et *al*., (1972) was used to assay for LDL – cholesterol concentrations of the blood samples.

$$\text{LDL}\left(\frac{\text{mg}}{\text{dl}}\right) = \text{Total Cholesterol} - \text{HDL} - \frac{\text{Triacylglycerol}}{5.0}$$

2.6.5. Statistical Analysis

The repeat measure analysis of variance (ANOVA) was used to compare similar mean values, and the group means were compared by Duncan's multiple range test (DMRT). The level of statistical significant was established at 5% probability level.

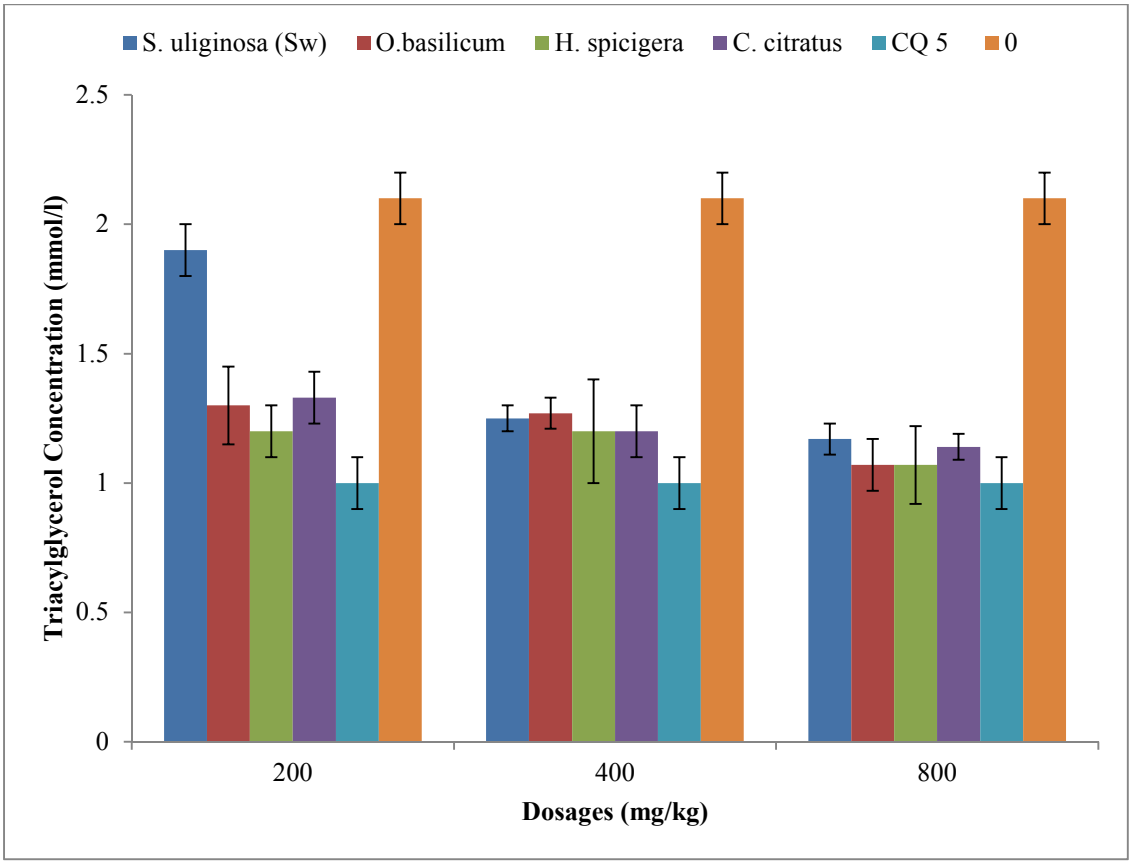
3. Results

The results of triacyglycerol concentrations for the treated and untreated mice are shown in figure 1. The results showed dose dependent significant decreases (P<0.05) in the level of triacyglycerol for all the extracts when compared to the negative control. There were significant difference (P<0.05) between the effects of the standard drug and the extracts of *S. uliginosa (Sw)* and *C. citratus* at all dosages used while the extract of *H. spicigera* at all doses and *O. basilicum* at only 800 mg/kg had no significant difference (P>0.05). The extracts of *C. citratus* at all doses marked significant differences (P<0.05) when compared to each other while the extracts of *H. spicigera* at all doses used, *O. basilicum* at 200 and 400 mg/kg and that of *S. uliginosa (Sw*) at 400 and 800 mg/kg did not show any significant difference (P>0.05) when compared to each other.

The results of total cholesterol concentrations for the treated and untreated mice are shown in figure 2. The results showed that there were significant reduction (P<0.05) in the level of total cholesterol for all the extracts at all varying doses except for *H. spicigera* when compared to negative control. There was a marked significant difference (P<0.05) between the effects of the standard drug and the extracts of *S. uliginosa (Sw*), *O. basilicum* at all given doses and that of *C. citratus* at 400 and 800 mg/kg while *H. spicigera* had no significant difference (P>0.05) at all doses used. The extract of *O. basilicum* at only 400 mg/kg and that of *S. uliginosa (Sw*) and *C. citratus* at only 200 mg/kg showed a significant difference P<0.05*)* when compared to other doses while the extract of *H. spicigera* at all the varying doses used did not cause any significant difference (P>0.05*)*.

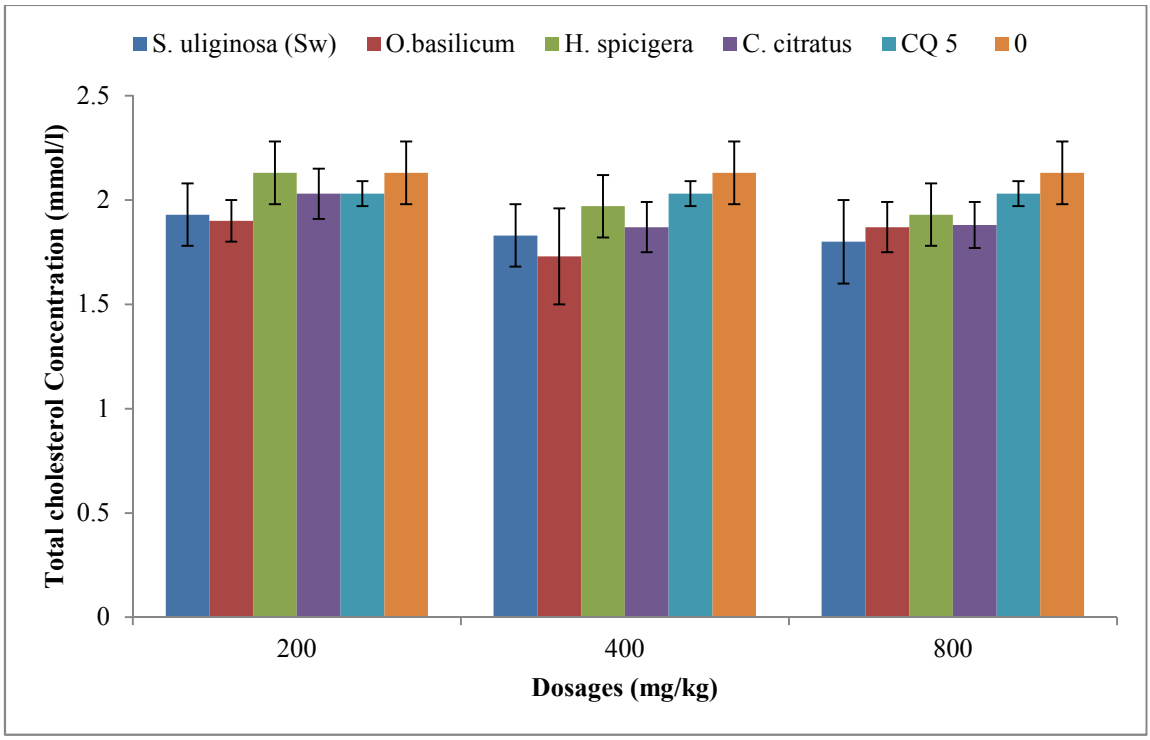
The results of HDL concentrations for the treated and untreated mice are shown in figure 3 .The results showed that there were significant elevation (P<0.05) in the level of HDL for all the extracts *at* all varying doses when compared to negative control. There was no significant difference (P>0.05) between the effects of the standard drug and the extracts of *S. uliginosa (Sw)* at varying doses used and *H. spicigera* at 200 and 400 mg/kg. However, there were marked significant difference (P<0.05) between the effects of the standard drug and the extracts of O. *basilicum* and *C. citratus* at varying doses used. The extracts *S. uliginosa (Sw)* at all given doses used and that of *C. citratus* at 400 and 800 mg/kg and *H. spicigera* at only 200 and 400 mg/kg had no significant differences (P>0.05*)* when compared to other doses while *O. basilicum* at all doses showed a significant difference (P<0.05).

The results of LDL concentrations for the treated and untreated mice are shown in figure 4. The results showed that there were significant reduction (P<0.05) in the level of LDL for all the extracts *at* all varying doses when compared to negative control. There was no significant difference (P>0.05) between the effects of the standard drug and the extracts of *S. uliginosa (Sw)* and *C. citratus* at varying doses used. However, there were marked significant difference (P<0.05) between the effects of the standard drug and the extracts of O. *basilicum* at varying doses used and *H. spicigera* at only 400 mg/kg. The extract *S. uliginosa (Sw) and C. citratus* at all given doses and that of O. *basilicum* at 400 and 800 mg/kg had no significant differences (P>0.05*)* when compared to each other while *H. spicigera* at all doses showed a significant difference (P<0.05).



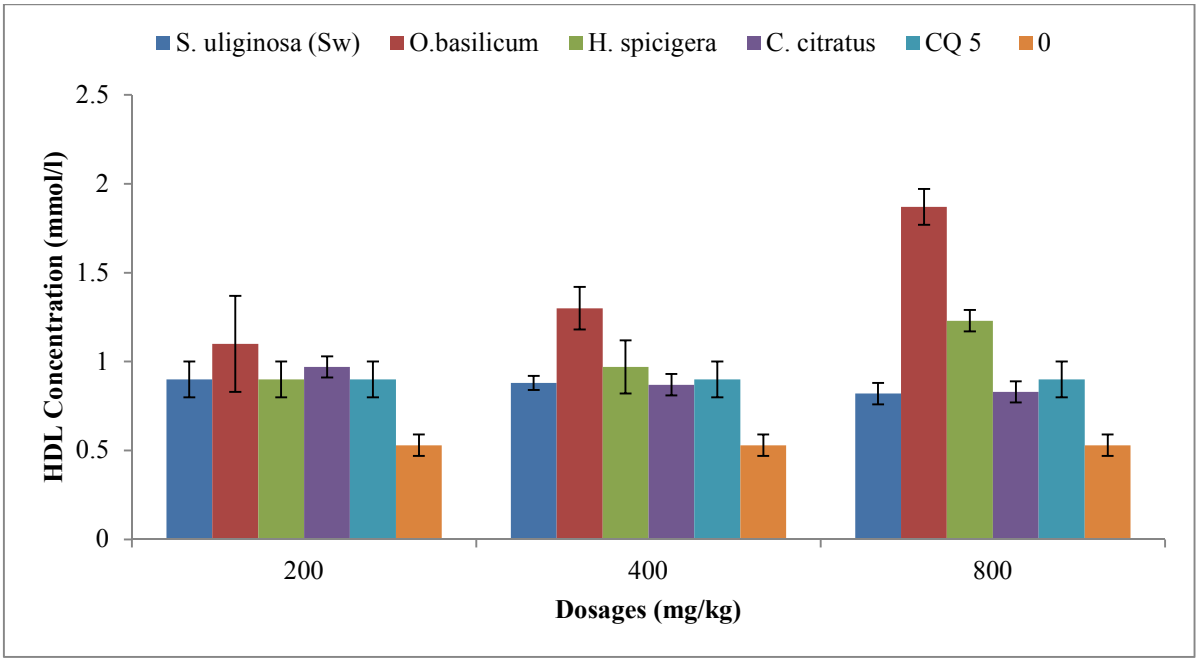
Bars bearing the same letters (dosage by dosage for each plant) are not significantly different from each other (P<0.05).

Figure 1. *The triacyglycerol concentrations (mmol/l) of the treated and untreated mice.*



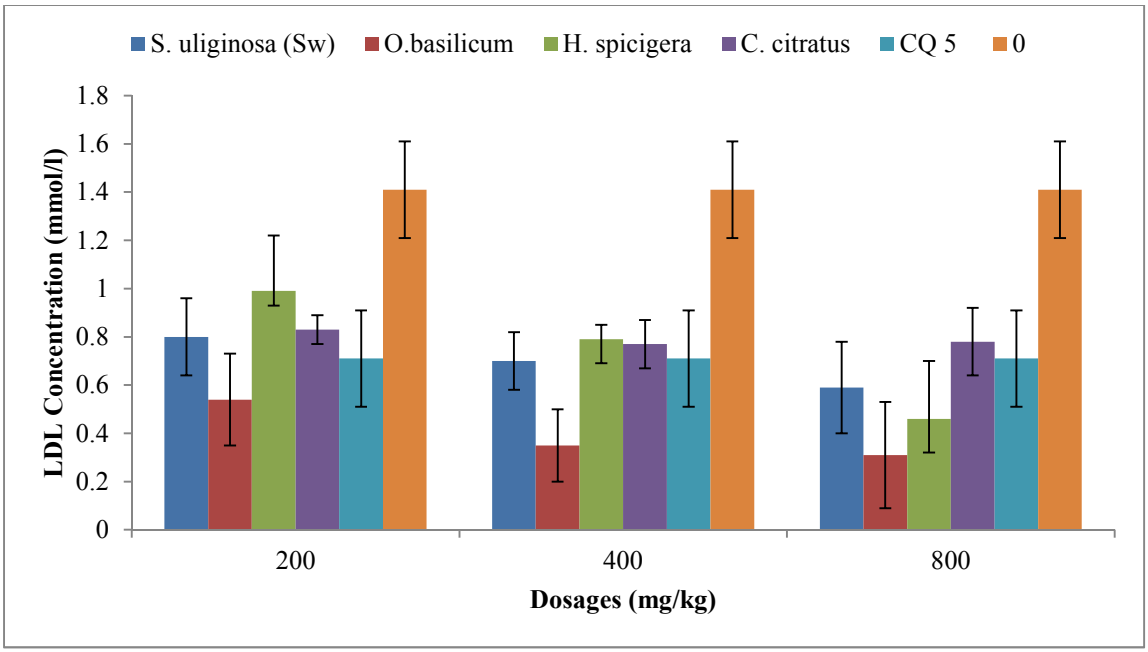
Bars bearing the same letters (dosage by dosage for each plant) are not significantly different from each other (P<0.05).

Figure 2. *The total cholesterol concentrations (mmol/l) of the treated and untreated mice.*



Bars bearing the same letters (dosage by dosage for each plant) are not significantly different from each other (P<0.05).

Figure 3. *The HDL Concentrations (mmol/l) of the treated and untreated mice.*



Key: Bars bearing the same letters (dosage by dosage for each plant) are not significantly different from each other (P<0.05).

Figure 4. *The results of LDL Concentrations (mmol/l) of the treated and untreated mice.*

4. Discussion

There was a general increase in physical activities of the mice treated with the extracts when compared with the parasitized untreated group. The results showed that the physical activities of the extracts - treated mice were better (more active) and this could be due to ameliorating effect of the plant extracts in malaria infection. The observed effect of the extracts may be attributed to some chemical components of the extracts such as alkaloids and saponins which have bactericidal and antispasmodic effects as well as antioxidant compounds which help to protect the animals against the damaging effects of reactive oxygen species imposed by malaria parasites (Achuba, 2005). This result disagrees with the report of Gregor (1997) who reported that the ethanol extract of *Ocimum basilicum* within one week of its administration caused a general decrease in physical activities of the animals. The reason(s) responsible for the differences are not clear but may suggest differences in species variation

and locations.

The results of lipid profile showed an increase in the concentration of total cholesterol, triacyglycerol and LDL-cholesterol and a decrease in HDL-cholesterol in untreated group compared to treated mice (Figure 1 - 4). This finding is consistent with those in other studies that showed elevated levels of lipoproteins like LDL, total cholesterol and triglyceride in patients suffering from malaria infection (Olusegun, 2013). Cholesterol is synthesized in the liver which happens to be the major site of plasmodium infection (Adekunle *et al*., 2007) and this raises some questions whether there is any relationship between the cholesterol synthesis by the liver and the plasmodium infection of the liver. Although, the parasite has ways that enables it to thrive and multiply using nutrients from the host, they still cannot synthesize majority of their own lipids and cholesterol *in vivo*. In view of this, one would have to expect that the serum lipid levels to be low compared with the treated group but in the infected mice the serum lipid levels were rather found to be higher compared with the treated group.

The higher concentrations of serum lipids in the infected group despite the requirement of lipids for the growth of the parasite, could be explained from the recent findings which suggest that the plasmodium genome contains genes encoding enzymes of phospholipids metabolism, allowing de novo synthesis of phosphatidylcholine via the kneddy pathway and necessitating only the uptake of the small choline molecule (Sowjanya *et al*., 2013). In addition, the genome of the parasite contains genes similar to those for type II fatty acid synthesis pathway. The protein products of these genes are located within the apicoplast and allow for the production of fatty acids, some of which are unique to the parasite (Krishna *et al*., 2013). Thus the parasite may be able to meet many of its lipid requirements from its own biosynthetic pathways.

The increase in the concentration of LDL-cholesterol is probably because of increased concentration of cholesterol. Increase in cholesterol, LDL and triglyceride levels during malaria infection have been reported to contribute to the pathogenesis of malaria and this could be dangerous to human health as it is capable of causing atherosclerosis if necessary treatment is not adopted. Lipoprotein has been reported to represent a major component of serum needed for the growth of the malaria parasite (Olusegun, 2013). LDL-cholesterol can be deposited on the walls of the artery leading to arthrosclerosis and other cardiovascular diseases (Mayers *et al*., 2007).

The concentration of HDL- cholesterol was either significantly or non - significantly lower in untreated animals. This lipid is synthesized by liver cells (Parks, 2002). Hence their lower concentration signifies liver dysfunction, resulting in the inability of liver to properly package the lipids (Sunmonu and Oloyede, 2007). HDL- cholesterol has higher molecular weight than other lipoproteins and transports cholesterol from the arterial walls back to the liver where it is used for biosynthesis. Therefore, its decrease in untreated group signifies increased chances of arthrosclerosis and vascular diseases (Tothe, 2005).

The observed higher concentration of HDL-cholesterol and triacyglycerol in the treated groups signified a better state of the liver and better cardiovascular health thus, indicating their promising protective role against CVD. There was also an increase in triacylglycerol concentrations in treated mice and this elevation may be attributed to initiation of fatty acid synthesis (Offor, 2011). These results obtained from lipid profile in this study could be attributed to the antioxidants (vitamin E, C and flavonoid etc) content of the plant extracts. Also, a number of studies have established that antioxidant vitamins prevent lipid oxidation and oxidative damage, thus impeding the progression of altherosclerosis (Achuba, 2005). More so, there is evidence that vitamin C and E increase HDL - cholesterol level and may also lower total cholesterol in the blood, thus reducing the risk of cardiovascular disease (Egbung *et al*., 2011).

5. Conclusion

It could therefore be inferred that the results obtained in the treated groups showed that *Spilanthes uliginosa (Sw), Ocimum basilicum, Hyptis spicigera and Cymbopogon citratus* could ameliorate Plasmodium induced changes in lipid profile and antioxidant enzymes, and such can be used in improving the cardiovascular health.

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