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## Ameliorating and Synergistic Effects of Ethanolic Extract of *Ocimum Sanctum* and Omega 3 Fatty Acids on Biochemical and Liver Function Indices of Mice

<sup>1</sup>Aisha, A., <sup>2</sup>Obaroh, I. O., <sup>2</sup>Attah, D. D., <sup>3</sup>Sani, K. A., <sup>4</sup>Andrews, O. A., <sup>1</sup>Jamilu M. A. and <sup>5</sup>Yusuf, H.,

<sup>1</sup> Department of Science Education, Waziri Umaru Federal Polytechnic, Birnin Kebbi. Kebbi state, Nigeria.

<sup>2</sup>Department of Animal and Environmental Biology, Kebbi State University of Science and Technology, Aliero, Nigeria.

<sup>3</sup>Department of Biological Sciences, Federal University Gusau, Zamfara State, Nigeria.

<sup>4</sup>Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.

<sup>5</sup>Department of Remedial Studies, Waziri Umaru Federal Polytechnic, Birnin Kebbi. Kebbi state, Nigeria.

**Corresponding Authors Email Address:** abubakaraisha1966@gmail.com or [sanikalgoabdurrahman@yahoo.com](mailto:sanikalgoabdurrahman@yahoo.com) **Phone no.:** 08097098712 or 08064446941

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### ABSTRACT

This study was carried out to investigate the ameliorating and synergistic effects of ethanolic extract of *Ocimum sanctum* and Omega 3 fatty acids on biochemical and liver enzymes assay of mice induced with *P. berghei*. Thirty mice were randomly distributed into six groups, five mice per group. Group A (Positive control, infected and untreated), Group B (100mg/kg of extract + 0.2ul Omega 3 fatty acids), Group C (200mg/kg of extract + 0.2ul Omega 3 fatty acids), Group D (300mg/kg of extract + 0.2ul Omega 3 fatty acids), Group E (0.2ul Omega 3 fatty acids) and Group F (0.1ml Artemether/lumefantrine serves as standard control). All groups were treated for 4 days, food and water were given in equal proportion. All parameters were analyzed using standard method of analysis. The qualitative phytochemical screening of *O. sanctum* showed high presence of volatile oils, and moderate presence of saponins, steroids, cardiac glycoside and alkaloids. Groups treated with the combination of *Ocimum sanctum* and Omega 3 fatty acids showed significant ( $P < 0.05$ ) increase in biochemical indices while Group F (standard control) and group A (infected and untreated) showed significant ( $P < 0.05$ ) decrease in the biochemical indices. The combination of *Ocimum sanctum* and Omega 3 fatty acids showed significant ( $p < 0.05$ ) decrease in enzymes assays at different concentrations, while the infected and untreated group shows significant increase in enzymes assay. This study indicates that synergy of *Ocimum sanctum* and Omega 3 fatty acids at different concentration showed ameliorating effects on liver damage, as indicated in the enzymes assay.

**Key word:** *Ocimum sanctum*, Omega 3 fatty acids, phytochemicals, biochemical, indices

### 1.0 INTRODUCTION

Control of malaria is complex due to drugs resistance strains. Some of the available drugs have been rendered ineffective due to the rapid spread of anti-malaria resistance by *Plasmodium* parasites. The high cost of anti-malaria drugs is another setback (Senior, 2005), thus there had been search for alternative drugs that is effective, cheap and readily available. Historically, medicinal plants have provided a source of inspiration for novel drugs compounds as plant derived medicines have made large contributions

to human health and well beings. The use of medicinal plants is increasing Worldwide; the World Health Organization (WHO, 2002) estimated that up to 80% of the world's population relies on plants for the treatment and control of their Health problem. Synthetic drugs gained popularity against green remedies because of their fast-acting effects, however chemically prepared drugs may act quickly, but they have side effects which affect the body negatively with time whereas, medicinal plants work in an

integrated approach with little or no adverse effects on the body (MacDonald, 2009).

*Ocimum sanctum* (Tulsi) belongs to the family Labiateae. It is an erect, sweet scented herb grown in tropical and subtropical including Nigeria and India (Bansod *et al.*, 2008). This plant has been evaluated pharmacologically for antimicrobial, immunomodulatory, anti-stress, anti-malaria, anti-inflammatory, antipyretic, anti-asthmatic, hypoglycemic, hypotensive and analgesic activities (Gupta *et al.*, 2002; Ahmed *et al.*, 2002). Also fish oils is rich in Polyunsaturated Fatty Acids (PUFAs), which has been documented to possess antimalarial and prophylactic properties (Lutgen, 2016). It has been reported that PUFAs causes marked in vitro growth inhibition of *Plasmodia*. The effect has been reported not only to be suppressive but also to be prophylactic in nature (Kumaratilake *et al.*, 2015).

Fatty acids are known as self-defensive agents in organism and possess various biological activities including anti inflammation (Schnmann, 2011). More recently it was confirmed that, this inhibition of *sporozoites* by long chain fatty acid applies to many parasitic pathogens of phylum *Apicomplexa* like *cryptosporidium Parvum*, *Toxoplasma gondii*, and *Plasmodium gallinaceum* (Schumann, 2011). The use of long-chain polyunsaturated fatty acid has been studied in the context of healing and tissue generation, mainly due to inflammatory, immune regulatory anti-oncogenic properties (Tiago *et al.*, 2015). Thus the need to investigate the synergistic and ameliorating effects of *Ocimum sanctum* and Omega 3 fatty acids on albino mice induced with *Plasmodium berghei*.

## 2.0 MATERIALS AND METHODS

### 2.1 Collection and Identification of Plants

*Ocimum sanctum* leaves were obtained within Kebbi State University of Science and Technology Aliero premises. The leaves were identified and authenticated by Dr Dramendra Singh at herbarium section of Biological Sciences, Botany Units, of the University, with voucher number KSUSTA/101.

### 2.2 Plant Preparation and Extraction

*Ocimum sanctum* leaves were washed with distilled water and shade-dried at room temperature for two weeks. Dried leaves were ground to fine powder by using pestle and mortar. Ethanolic extract was prepared by soaking 200g of ground *Ocimum sanctum* leaves into 2Litres of ethanol for 48hours. Afterward the resulting mixture was filtered using filter paper, the filtrate was further concentrated in a water bath at 45 °C to obtain a jelly-like substances. Extract was stored in a refrigerator until when needed.

### 2.3 Acquisition of Experimental Animals and Parasite Stock

Fifty mice of weighting between 19 -30g were obtained from Usmanu Danfodiyo University Sokoto Zoological garden. *Plasmodium berghei* (NK-65) strain was obtained from Biochemistry laboratory, Usmanu Danfodiyo University, Sokoto.

### 2.4 Mode of Infection

Blood was taken from donor albino mice previously infected with *Plasmodium berghei* (NK-65) strain, in which the parasitaemia is high and rising (30% or more infected red cell). The number of parasite per 500 red cells was estimated. From the donor mice, 1ml of blood was taken and diluted with 5ml of phosphate buffered, such that 0.1 ml contained standard inoculum of  $1 \times 10^7$  parasites infected red cells. From the pool of acclimatized mice thirty mice were randomly selected and were inoculated intraperitoneally from the same source to avoid variability in parasitaemia (Banyal *et al.*, 1991).

### 2.5 Qualitative Phytochemical Screening

The extract was evaluated for the presence of saponin, tannin, flavonoids, alkaloid, anthraquinons, volatile oil, steroid, cardiac glycoside. Analysis was done according to the method described and reviewed by Prashant *et al.* (2011).

### 2.6 Experimental Designs

The study was carried out using Peter's 4 day – Test (Peter, 1970). Thirty mice were randomly distributed into six groups, five mice per group. Group A (Positive control) Group B received 100mg/kg of extract + 0.2ul Omega 3

fatty acids, Group C received 200mg/kg of extract + 0.2ul Omega 3 fatty acids, Group D received 300mg/kg of extract + 0.2ul Omega 3 fatty acids. Group E received 0.2ul Omega 3 fatty acids and Group F received 0.1ml Artemether/lumefantrine(Standard control). All groups were treated for 4 days, and given food and water of equal proportion.

**2.7 Biochemical Analysis**

Blood samples were collected from experimental rat and subjected to serum analysis to obtain information on target organs. The blood was put into 5ml (BD Vacutainer® SST™) plain tubes and allowed to clot at room temperature. The samples were centrifuged at 3000 g for 10 min to separate the plasma and sera into Eppendorf tubes, labeled and stored in laboratory freezer at -80°C until analysis. Each parameter was determined as follows:

**2.7.1 Determination of Protein**

Test tubes were labeled as blank standard and samples. Spectrophotometer was set to zero with 1.0ml saline in a test tube; the serum blank was read and its absorbance recorded. Working reagents of 1.0ml (total protein reagent and total protein standard) and 0.02ml of serum sample were deep into each test tube and mixed. The mixtures were incubated for ten (10) minutes at room temperature (25°C). The absorbance of standard and that of the samples were measured against the blank at 540nm and recorded (Domas and Biggs (1997). Total protein (TP) concentration was calculated using equation (1) below:

**TP Conc (mg/dl) =**

$$\frac{\text{Abs of sample}}{\text{Abs of Std}} \times \text{Conc. of std} \dots\dots\dots (1)$$

Where:  
Abs = Absorbance, Conc = Concentration,  
Std = standard

**2.7.2 Determination of Albumin**

Serum albumin was determined colorimetrically using bromocresol green (BCG): Three test tubes

were labeled as test, standard and blank respectively. Serum (0.1 ml) was added to the test, 0.1 ml of standard albumin was added to standard and 0.1 ml of water to the blank. BCG (0.8 ml) reagent was then added to each of the test tubes. Subsequently, the tubes were incubated at 37°C for 15 minutes and the absorbance of the test and standard were read at 640 nm against the blank (Bartholomew and Delany,1998). Albumin concentration was calculated using equation (2) below:

**Conc. of Albumin (mg/dl) =**

$$\frac{\text{Abs of sample}}{\text{Abs of Std}} \times \text{Conc. of std} \dots\dots\dots (2)$$

Where:  
Abs = Absorbance, Conc = Concentration,  
Std = standard

**2.7.3 Test for Alkaline phosphatase (ALP)**

Test tubes were labeled as blank, standard and samples. For each test tube 0.5ml alkaline phosphatase substrates was pipette and left for three 3 minutes at 37°C to equilibrate, 0.05ml of standard control and serum sample were dispense in to the respective test tubes and then gently mix together and incubated for ten (10) minutes. Alkaline phosphatase colour developer measuring 2.5ml was added to the mixtures and gently mixed. The spectrophotometer was zero with the blank and its observance recorded. The absorbance of the standard and that of the samples was measured against the blank at 620nm and recorded (Roy, 1970). Alkaline phosphatase concentration was calculated using equation (3) below;

**ALP Conc (mg/dl) =**

$$\frac{\text{Abs of sample}}{\text{Abs of Std}} \times \text{Conc. Of std 50 (U/l)} \dots\dots\dots (3)$$

Where:  
Abs = Absorbance, Conc = Concentration,  
Std = standard

**2.7.4 Measurement of Serum Aspartate Aminotransferase (AST) Activity**

Three test tubes, labeled blank, standard and test were set up in triplicates; 5ml of the buffered

substrate were pipetted into the test tube labeled 'test' and incubated in a water bath at 37 °C for 5minutes, 0.1ml of serum was added, mixed and returned into the water bath for additional 60minutes. Just before 60min was due the following was pipetted into the blank and standard test tubes; for the substrate, 0.1ml distilled water and 0.5ml buffered substrate. For the blank 0.1ml distilled water, 0.3ml buffered substrate and 0.2ul standard pyruvate solution. At exactly 60mins the incubated test tubes were removed and 0.5ml of coloured reagent was added to all the tubes and allowed to stay at room temperature for 20mins. Five millilitre of 0.4M NaOH was added to each of the test tubes and allowed to stand for additional 5 minutes. The absorbance of the standard and test was read against the blank at 505nm. The enzyme activity, which was expressed in U/l were obtained from a calibration curve that was constructed (Reiman and Frankel, 1957).

**2.7.5 Measurement of Serum Alanine Aminotransferase (ALT) Activity**

Alanine Transaminase activity was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine according to Reitman and Frankel, (1957). Two test tubes were set up labeled T1 (reagent blank) and T2 (test sample) .T1 contained 0.01 mL distilled water and 0.50 ml Randox buffer solution, while T2 contained 0.01 ml serum and 0.50 ml Randox buffer solution, the contents were mixed and incubated for 30 minutes at 37oC to each tube of was added 0.05 ml of Randox 4–dinitrophenylhydrazine solution and the contents were mixed and allowed to stand for 20 min at 25oC in a water bath. Then 5 ml of sodium hydroxide solution was added to each of the tubes. The contents were mixed, and after 5 min their absorbance values were read at 546 nm against the reagent blank in the spectrophotometer and the activity of the enzyme read off from the table.

**2.7.6 Estimation of Serum Bilirubin Concentration**

The serum bilirubin concentration was determined using Randox Test Kits (Randox Laboratories Ltd, Crumlin, England, UK) Malloy and Evelyn, (1937).

**Total Bilirubin (TB)**

Two tubes were set up labeled T1 (sample blank) and T2 (test sample). T1 contained 0.20 mL Randox sulphanilic acid Reagent, 1.0 ml Randox caffeine solution and 0.2 ml of serum, while Randox sulphanilic acid Reagent 0.05 ml Randox sodium nitrite solution, 1.0 ml of Randox titrate solution their content were mixed and allowed to stand for 30 min at 25° C in a water bath, before reading the absorbance (Abs) at 578 nm against the sample blank in a spectrophotometer. Total Bilirubin concentration was calculated using equation (4) below:

**TB Conc (mg/dl) =**

$$\frac{\text{Abs of sample}}{\text{Abs of Std}} \times \text{Conc. of std} \dots\dots (4)$$

Where:

Abs = Absorbance, Conc = Concentration, Std = standard

**Direct Bilirubin (DB)**

Two test tubes were set up labeled T1 (sample blank) and T2 (test sample). T1 contained 0.20 m L Randox sulphanilic acid Reagent, 2.00 ml of 0.9% sodium chloride solution and 0.20 ml serum. The contents were mixed and allowed to stand for 5 min at 25°C, before reading the absorbance (Abs) at 546 nm against the sample blank in a Spectrophotometer. Direct Bilirubin concentration was calculated using equation (5) below:

**DB Conc (mg/dl) =**

$$\frac{\text{Abs of sample}}{\text{Abs of Std}} \times \text{Conc. of std} \dots\dots (5)$$

Where:

Abs = Absorbance, Conc = Concentration, Std = standard

**2.7.7 Statistical Analysis**

All numerical data were subjected to statistical analysis using the statistical package for social sciences (SPSS) 18.0. Mean± SD were calculated for each data and significant differences between the group were evaluated using One way

TP Conc (g/dl) =

TP Conc (g/dl) =

Analysis of Variance (ANOVA) Post-test analysis were done using the Duncan multiple Range at 95% level of significance.

### 3.0 RESULTS

#### 3.1 Qualitative Phytochemical Analysis of *Ocimum sanctum* leaf extract

Table 1 shows the results of qualitative phytochemical analysis of *Ocimum sanctum* leaf extract. The result showed high presence of volatiles oil, moderate presence of steroids, Alkaloids Saponin, Cardiac Glycoside, Low Present of Flavonoids while Free anthraquinones and Tannin were absent.

#### 3.2 Biochemical Indices of Experimental Mice

The results on the biochemical indices are presented in Table 2. The highest value for total Protein was observed in Group C (200mg/kg + 0.2ul Omega 3 fatty acids) with a mean value of  $5.23 \pm 0.25$  mg/dl, while the lowest was observed in Group F (standard control) with a mean value of  $3.11 \pm 0.48$  mg/dl. The highest value for Total Albumin was observed in Group E (0.2ul Omega 3 fatty acids) with mean value of  $1.99 \pm 0.54$  mg/dl, while the lowest value was observed in Group F (Standard control) with mean value of  $0.98 \pm 0.46$  mg/dl. The highest value for Total Bilirubin was observed in Group B (100mg/kg + 0.2ul Omega 3 fatty acids) with a mean value of  $2.22 \pm 0.12$ , while lowest value was observed in Group F (Standard control) with mean value of  $1.79 \pm 0.28$  mg/dl. The highest value for Direct Bilirubin was observed in Group D (300mg/kg + 0.2ul Omega 3 fatty acids) with a mean value of  $1.55 \pm 0.24$  mg/dl, while lowest value was

observed in Group in B (100mg/kg + 0.2ul Omega 3 fatty acids) with mean value of  $0.67 \pm 0.08$  mg/dl. Analysis of variance showed significant difference ( $P < 0.05$ ) when the highest values obtained were compared with lowest values.

#### 3.3 Liver Enzymes assay of Experimental Mice

The results on the Liver enzymes assay of experimental mice is presented in Table 3. The highest value for ALP and AST were both observed in Group A (Positive control) with mean value of  $224.74 \pm 18.45$  U/l and  $234.33 \pm 6.58$  U/l, while the lowest were also both observed in Group B (100mg/kg + 0.2ul Omega 3 fatty acids) with mean value of  $123.41 \pm 33.33$  U/l and  $35.52 \pm 3.56$  U/l. The highest value for ALT was observed in Group A (Positive control group) with mean value of  $92.44 \pm 10.76$  U/l, while the lowest value was observed in Group B (100mg/kg + 0.2ul Omega 3 fatty acids) with mean value of  $54.15 \pm 3.45$  U/l. Analysis of variance showed significant differences ( $P < 0.05$ ) when the highest values obtained were compared with the lowest values.

**Table 1: Qualitative phytochemical analysis of *Ocimum sanctum* Leaf**

Composition	Result
Alkaloids	++
Saponin	++
Tannin	-
Steroids	++
Flavonoid	+
Free Antraquinone	-
Volatile oils	+++
Cardiac Glycoside	++

Highly Present +++, Moderately Present ++, Low +, Absent -

**Table 2: Biochemical indices of Experimental Mice**

Grps	Doses(mg/kg)	Total protein (mg/dl)	Total Albumin (mg/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
A	Positive control	$3.42 \pm 1.02^a$	$1.56 \pm 0.67^{ab}$	$1.99 \pm 0.15^{cd}$	$0.85 \pm 0.04^a$
B	100mg/ + 0.2ul Omega 3fatty acids	$4.36 \pm 1.11^{ab}$	$1.05 \pm 0.24^a$	$2.22 \pm 0.12^d$	$0.67 \pm 0.08^a$
C	200mg/kg + 0.2ul Omega 3 fatty acids	$5.23 \pm 0.25^b$	$1.01 \pm 0.11^a$	$1.48 \pm 0.13^{ab}$	$0.82 \pm 0.19^a$

D	300mg/kg 0.2ul Omega 3 fatty acids	5.06 ± 0.25 <sup>b</sup>	1.52 ± 0.63 <sup>ab</sup>	1.81 ± 0.29 <sup>bc</sup>	1.55 ± 0.24 <sup>b</sup>
E	Omega 3 fatty acids 0.2ul	4.80 ± 0.32 <sup>b</sup>	1.99 ± 0.54 <sup>b</sup>	1.35 ± 0.21 <sup>a</sup>	1.49 ± 0.43 <sup>b</sup>
F	Standard control (ACT) 0.2ul	3.11 ± 0.48 <sup>a</sup>	0.98 ± 0.46 <sup>a</sup>	1.79 ± 0.28 <sup>bc</sup>	1.07 ± 0.28 <sup>ab</sup>

Data are presented as mean ± SD (P ≤ 0.05)

### Table 3: Liver Enzymes assay of Experimental Mice

Data are presented as mean ± SD, P ≤ 0.05

ALP= Alkaline Phosphatase, AST= Aspartate Amino Transferase, ALT=Alanine Amino transferase.

ALP= Alkaline Phosphatase, AST= Aspartate Amino Transferase, ALT=Alanine Amino transferase.

## 4.0 DISCUSSION

The study reveals the ameliorating effects of the synergy between *Ocimum sanctum* extract and omega3 fatty acids. It was observed that the ameliorating of both synergistic extracts of

3.8-4.8mg/dl) respectively reported for ALP, ALT, Total Protein and Albumin on Rat Biochemical Standard Reference range (2014). The values of Total Bilirubin and Direct bilirubin were higher than the range of values (0.2-

Groups	Doses (mg/kg)	ALP(U/l)	AST(U/l)	ALT(U/l)
A	Untreated Infected	224.74 ± 18.45 <sup>c</sup>	234.33 ± 6.58 <sup>d</sup>	92.44 ± 10.76 <sup>b</sup>
B	100mg/kg +0.2ul Omega 3 fatty acids	123.41 ± 33.33 <sup>a</sup>	35.52 ± 3.56 <sup>a</sup>	54.15 ± 3.45 <sup>a</sup>
C	200mg/kg+ 0.2ul Omega 3fatty acids	135.42 ± 12.43 <sup>a</sup>	43.21 ± 4.89 <sup>a</sup>	59.60 ± 7.67 <sup>a</sup>
D	300mg/kg+ 0.2ul Omega 3 fatty acids	149.43 ± 24.01 <sup>ab</sup>	55.35 ± 7.21 <sup>b</sup>	58.07 ± 5.66 <sup>a</sup>
E	0.2ul Omega 3 fatty acids	132.40 ± 14.16 <sup>a</sup>	58.22 ± 4.56 <sup>b</sup>	61.24 ± 13.20 <sup>a</sup>
F	Standard control (ACT)	187.330 ± 21.23 <sup>bc</sup>	90.18 ± 5.55 <sup>c</sup>	66.64 ± 14.48 <sup>a</sup>

*Ocimum sanctum* and omega3 fatty acid are dose dependent at different concentrations. This effects could be attributed to the presence of alkaloids, saponins, steroids, volatiles oil cardiac glycosides and flavonoids in *Ocimum sanctum* extract, previously reported by Khan and Bhatia, (2003), Chiag *et al.*, (2005), Matur *et al.* (2009), also reported similar composition.

Moreover, the constitutes could serve as immune modulators, antioxidants and inhibitors of haema-polymerisation as reported by Aherne *et al.* (2007). Saponns and polyphenol have been associated with anti-anemic activities (Yakubu *et al.*, 2005). Saponin serves as anti-biotic in fighting infection and microbial invasion, furthermore it increases intestinal permeability (Gbadomosi *et al.*, 2011).

This result shows significant increases in liver enzymes (ALP, AST, ALT) in untreated infected groups and significant decreases of ALP, AST, ALT in treated group. Comparably, the values of ALP, ALT, Total Protein and Albumin obtained in this study were lower than the range of values (56.8- 128U/l, 17.5 - 30.2U/l, 5.6-7.6mg/dl and

0.55mg/dl) reported for Bilirubin on Rat Biochemical Standard Reference range (2014). While the values of AST obtained in this study were with the standard range of values (45.7 to 80.8U/l) reported on Rat Biochemical Standard Reference range (2014) except in Groups A (Untreated Infected), Group F (Standard control (ACT) which were high and group B which was lower than reference range.

Measurements of the liver function in body fluids play a significant role in disease investigation and diagnosis and detection of tissue damage (Victoria *et al.*, 2010). The liver enzymes assay (ALP, AST and ALT) are indicators of liver diseases they are biomarker of organ functions, their elevation in serum therefore suggest tissue damage or organ dysfunction (Igile,1995; Merck, 1998; Olivera *et al.*, 2004; Arise *et al.*, 2012). This shows the synergistic potency of both ethanolic extract of *Ocimum sanctum* and omega 3 fatty acid to recover liver damaged by malaria. PUFAs possess well documented antimalarial and prophylactic properties (Lutgen, 2016). The

effect is not only suppressive but also prophylactic (Kumaratilake *et al.*, 2015).

## 5.0 CONCLUSION

Conclusively this study showed that extract of *Ocimum sanctum* and omega 3 fatty acids possess ameliorating effects on biochemical indices of rat induced with *Plasmodium berghei*. The synergy has the potential of restoring liver damage as observed in biochemical indices and liver enzymes assay. The modulation may be attributed to PUFAs and the active constituent in the plant extract.

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