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Nutritional and Immunomodulatory Activities of Extra Virgin Olive Oil in the Treatment of Diarrhoea in Albino Rats Infected with *Escherichia Coli* 0157:H7

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ABSTRACT

Nutrition refers to the study or intake of food considered in relation to the body's dietary needs while immunomodulation refers to changes in the activity of the immune system caused by factors inhibiting the functions of the immune system, hence, studies aimed at modifying the immune response to prevent infections. In this present study, the effectiveness/imunomodulatory effect of extra virgin olive oil (EOO) supplementation in the diet of experimental animals (albino rats) infected with *E scherichia coli* 0157:H7 to cause diarrhoea was studied. Effects of EOO on weight gain and haematological profile of mice was also monitored. *E. coli* counts were performed in both animal stool (feces) and urine. For the experiment, 16 albino rats were assigned at random to four (4) groups designated as A, B, C, and D, (four animals per group) and all were aged between 4-6 weeks. The rats were acclimatized for ten (10) days and subsequently monitored for seven (7) days (experimental period). The result of the experiment showed that the infectivity dose of *E. coli* 0157:H7 in albino rats was 1.7×10^3 Cfu/ml. The animals in groups A-C were inoculated orally (1.7×10^3Cfu/ml/mouse) while animals in group D were used as control. Loss in weights occurred in animals when infection set. The *E. coli* 0157:H7 infection caused diarrhoea which was treated with extra virgin olive oil (A), chloramphenicol (B), Not treated at all (C). The treatment with extra virgin olive oil was however not as effective as the standard antibiotic (chloramphenicol) in infected animals. The haematological profile of the rats revealed high Red Blood Cell counts (RBC), Packed Cell Volume (PCV), Heamoglobin (Hb), low White Blood Cell counts (WBC) and WBC differential counts revealed higher lymphocyte counts, low neutrophil and monocyte counts, normal counts for eosinophils and basophils when compared with the control group (D). Also the only microorganisms isolated from the extra virgin olive oil (EOO) were identified as *Staphylococcus epidermidis*. The results obtained in this study has shown that extra virgin oil has antibacterial property and that it was able to cure diarrhoea caused by E. coli 0157:H7 in albino rats.

Keywords: Nutritional, Immunomodulatory, Olive Oil, Albino Rats.

INTRODUCTION

Nutrition is the study or intake of food considered in relation to the body's dietary needs (i.e considered a critical determinant of immunocompetence) and a key to developing/maintaining good health, a state of complete physical, mental and social well being. Immunomodulation on the other hand is defined as changes in the activity of the immune system caused by factors that can activate or inhibit its functions and can be achieved through dietary components. So, immunomodulation has been extended for the purposes of therapeutic interventions aimed at modifying the immune response to prevent infections.

According to current understanding, the immune response of both humans and animals may be influenced by several essential nutrients which modify the immune system functions. Infact it is generally accepted that many of the important human infections have been associated with nutritional deficiency (Chandra 1996).Therefore the interaction between certain nutrients and immunity exert a crucial role that should be analyzed from a clinical and biological point of view. Lipids being one of these nutrients exert great influence on the genesis of chronic diseases, such as obesity, atherosclerosis and other cardiovascular diseases (Alfaia *et al*., 2009). This influence however, may be a consequence of one or several factors but in general, the main event may be associated with changes in cell membrane due to dietary fatty manipulations, this is because lipids (fatty acid) may be incorporated into the plasma membrane after administration so that the composition of lipid in the cell structure will reflect the composition of dietary lipids (Clamp *et al*., 1997). Because of this incorporation also, the phospholipid profiles associated with plasma membrane of lymphocytes, monocytes and other cells may be altered by dietary lipids (Chapkin and Camichael, 1990).

Olive oil is an important dietary lipid, expressed from olive fruits. Olive oil has been used in traditional medicine for several thousands of years in countries of the Mediterranean basin. It is available in a variety of grades reflecting the degree to which it has been processed which makes it widely available for cooking, cosmetics, pharmaceuticals as well as therapeutic uses. Olive oil is found in many religions and cultures, termed anointing oil used during special ceremonies, e.g baptism in the christian church, for ordination of priesthood as well as consecration of the materials in the tabernacle (Exodus 30:26, 29), to anoint the dead in some Greek and Jewish culture and applied to the sick and wounds (Psalm 109:18; Isaiah 1:6) because of its healing properties. According to history, olive oil is the major culinary fat in countries where olives are grown and constituting parts commonly referred to as 'Mediterranean diet'. The nutritional and modulatory benefits of olive oil supplementation as reported by many investigations in both humans and animals is attributed to the fact that it is made up of monounsaturated fatty acids (MUFA) and functional bioactive such as carotenoids, tocopherols, phospholipids with multiple biological activities and thus provide the purest form of fat in which bacteria cannot survive. Monounsaturated fats are found in excellent amounts in olive oil which help to lower the LDL (low density lipoproteins) but promote the HDL (High density lipoprotein) preventing coronary heart diseases, hypertension, cancer, autoimmune disorders and also presenting results for inflammatory responses (Simopoulous, 2008). Olive oil is use in food preparation or as condiments because it enhances the taste of many foods and less quantity is required to add its distinctive flavor to food.

Though it is unusual to think about culinary oil having a therapeutic function but the strength of dietary lipids as documented by research works are through a number of mechanisms to produce a potential effect on the immune system functions (Mc Murray *et al.*, 2000). The modulation of immune system may occur by alteration of membrane fluidity, lipid peroxide formation (Allard *et al*., 1997) and regulation of gene expression (Jump and Clarke, 1999). Recent studies have however reported the effects of several free fatty acids on induction of in vitro cultures and ability to alter the survival of animals fed diet containing oil

and infected with a pathogenic bacterium by producing a modification of resistance to microorganisms with reduction of symptoms when certain parameters were tested.
This study is expected to provide information on extra virgin olive oil (EOO) for treatment of diarrhoea caused by *Escherichia coli* 0157:H7 infection. Olive oil is generally available commercially; its wide usage all over the world without prescription for the treatment of various ailments and claim that it is a good therapeutic agent needs to be confirmed scientifically.

MATERIALS AND METHODS

Sample Collection

The extra virgin olive oil used was bought from Akure central market and taken to Department of Microbiology laboratory for analysis.

Preparation of Media

The media used are Nutrient agar, Potato dextrose agar, Eosine methylene blue, and Nutrient broth. They were all prepared according to the manufacturer's instruction before sterilization at 121^0C for 15 minutes in an autoclave. The media were allowed to cool to a temperature of about 45^0C before they were gently aseptically into sterile Petri dishes and allowed to solidify at room temperature.

Experimental Animals

A total of 16 male albino rats aged between 4-6weeks old were used for this study. The animals were purchased at the laboratory animal section of the Federal University of Technology Akure, Ondo State. The animals were acclimatized for 10days before the commencement of this experimental work i.e they were fed with broiler starter product purchased from the market in Akure, Ondo State. The feed and water was given to the animals (rats) twice daily. They were caged separately (four animals per cage). The cage was cleaned daily using soap and dettol in order to maintain a high level of hygiene.

Experimental Design

After acclimatization, the animals (rats) were fasted for about 24hours and were assigned to four (4) treatment groups designated as A, B, C and D. Rats in groups A-C were infected with the organism *E. coli* 0157:H7 while those in group D were not infected and so, D served as the control group after which they were permitted access to food and water. Provision was made at the bottom of the cage to aseptically collect the faeces for analysis.

A- Infected and treated with feed+ one milliliter (1ml) of olive oil mixed once per day.
B- Infected and treated with feed+ one milliliter (1ml) of dissolved chloramphenicol (50mg) once per day.
C- Infected and treated with feed alone
D- Not infected at all (control group)

Weighing of Animals

The weight of the animals were taken daily throughout the pre and post ingestion period using the method of Momoh *et al*., (2012). Each of the four (4) rats in a group was numbered using a permanent marker in order to avoid mix up. The weight was taken early in the morning using the Kerro weight measurement model.

Collection of blood samples from Experimental Animals

The 16 albino rats used for this study were sacrificed by cervical dislocation and the blood samples were collected into EDTA bottles for haematological tests.

Preparation of Infectivity Dose for *E.Coli* 0157:H7 Strain

Pure bacterial strains of *E.coli* 0157:H7 was obtained (stock) and grown in about 100ml of Nutrient broth at 37^0C for 24hours. The broth culture was poured into a sterilized centrifuge tube and centrifuged at 3000rpm for 5minutes. The culture was decanted and the cells were harvested and washed by re-suspending the cells in sterile distilled water and again centrifuged. This procedure was repeated for about three times. The stock solution of *E.coli* 0157:H7 obtained was subjected to serial dilutions using the method of Willey *et al* (2008) with slight modifications. Sterile tubes were arranged and 9ml of sterile distilled water was dispensed into 5 sets of testtubes. 1ml of the stock solution was introduced into the first tube making 1:10 dilution, this procedure was repeated for the remaining four (4) testtubes and 1ml of each dilution was cultured using pour-plate method on MacConkey agar at 37^0C for 24hours. Visible colonies were counted and estimated according to the dilution factor. A set of new animals aged between 4-6 weeks were used for this experiment. They were 15 (fifteen) in number and were also divided into five (5) groups. Each of the three animals in the five groups was challenged (infected) orally with 1ml of the different corresponding dilutions and the infective dose was found to be 1.7×10^3Cfu/ml Having obtained this, the experimental animals in groups A-C were infected with 1ml of this dose (1.7×10^3 Cfu/ml/mouse) and they were observed for 7days.

Isolation of Microorganism from Sample

Isolation was done using the pour plate method described by Fawole and Oso (2004). One ml of the Goya extra virgin olive oil was aseptically measured into a sterile Petri dish and pour-plated using Nutrient agar and Potato dextrose agar respectively. The mixtures were properly homogenized and allowed to solidify. The plates were incubated at 37^0C for 24hours (NA) and 25^0C for about 72 hours (PDA) before they were examined for growth.

Isolation from Stool and Urine of Animals

About 1g of the animal stool (faeces) was weighed from each of the four groups into 9ml of sterile distilled water and was serially diluted to power 4. One ml was aseptically measured from each of the four diluted samples into sterile petridishes and pour-plated using Nutrient agar. The plated samples were incubated at 37^oC for 24 hours. These fecal samples were collected on days 1, 4 and 7 after infection.

About 0.5 ml of the animal urine was collected from the groups into 9.5 ml of sterile distilled water each and was serially diluted to power 4. One ml was aseptically measured from each of the four diluted samples into sterile Petri dishes and pour-plated using Nutrient agar. The plates were subsequently incubated at 37^0C for 24hours.

Identification of Isolates

a) Sugar Fermentation

This test was carried out in order to know the ability of the isolates to ferment sugars with or without the production of gas. Each of the sugars (lactose, sucrose, glucose, fructose, manitol) was prepared and dissolved in 10% solution of sterile broth. Phenol red indicator was added to the broth and about 10ml was dispensed into test tubes from each of the sugar solution. The test tubes were sealed using cotton wool and foil paper and were sterilized in an autoclave at 121^0C for 15minutes. The tubes were then inoculated with the isolates and were incubated at 37^0C for about 72hours. Change in the color was observed while the gas production was indicated by presence of air space in the Durham tubes.

b) Biochemical Tests

These include catalase reaction, coagulase test, starch hydrolysis, indole test, oxidase test, urease test, motility tests and Gram staining reaction.

Hematological Assay

Haematological assay, which includes analysis for Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV), White Blood Cell Count (WBC), Haemoglobin Concentration (HB), Red Blood Cell Count (RBC) and WBC Differential Count (Lymphocytes, Neutrophils, Monocytes, Eosinophils and Basophils) were carried out using standard methods described by Momoh *et al*. (2012).

Statistical Analysis

Statistical analysis of data was carried out using analysis of variance (ANOVA) and significant difference between the treatment means was determined at 95% confidence interval using Duncan's Multiple Range Test for the estimation of means.

RESULTS

The animals after they have been infected with *Escherichia coli* 0157:H7 showed signs and symptoms such as dullness, loss of appetite, falling of fur and passage of watery stool within 24 hrs after infection.

Weight of Animals

The weight of the animals in each of the groups (A-D) increased gradually as expected during the period of acclimatization. All *Escherichia coli* 0157:H7 infected animals (A-C) demonstrated a drop in weight from day 0 to day 1 of infection (between 1.1-1.8g). The animals in groups A and B however started to return to their respective pre-infection weights after day 2 of their various treatment plans (i.e feed + olive oil for group A and feed + chloramphenicol for group B). Animals in group C were infected and not treated, they had significant weight reduction and they never returned to their pre-infection weights throughout the experiment period. Animals in group D were not infected with the organism *Escherichia coli* 0157:H7 (control group) so their body weight was a constant trend (i.e increased gradually) all through the experiment period. This is shown in figure 1 representing the mean weight of all the albino rats in each of the groups.

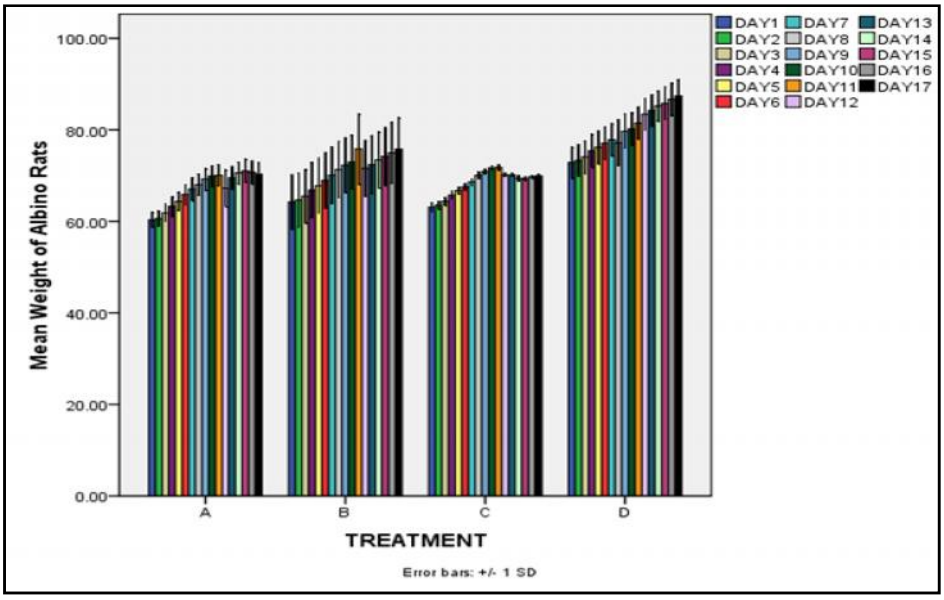

Figure 1: showing the mean weight of the Albino rats in the four groups all through the experimental period

KEY: A:Infected and treated with olive oil, B:Infected and treated with chloramphenicol, C: infected and not treated, D:Not infected (Control group).

Observations

The result of the infectivity dose test on the animals used showed that infectivity dose was 1.7×10^3Cfu/ml The duration for infection to set in (i.e the time overt signs and symptoms of infection were noticed on the animals) was within the range of 24-48hours. This result is shown in table 1. The overt signs and symptoms after infection and through the time of the research are shown in table 1. Animals displayed such manifestation of disease as weight loss, dullness, loss of appetite, falling of fur and the passage of watery stool.

Table 1: Signs and symptoms displayed by the infected rats

Days	Group A	Group B	Group C	Group D
1	A, NSI, FS	A, NSI, FS	A, NSI, FS	A, NSI, FS
2	D, US, LA	D, US, LA	D, US, LA	A, NSI, FS
3	D, US, LA	D, FS,	D, US, LA	A, NSI, FS
4	D, US,	A, FS	D, US, FF	A, NSI, FS
5	A, US	A, FS	D, US, FF	A, NSI, FS
6	A, FS	A, FS, NSI	A, FS, FF	A, NSI, FS
7	A, FS, NSI	A, FS, NSI	A, FS,	A, NSI, FS

KEY: A-Active, D-Dullness**,** LA-Loss of appetite**,** FS-Formed stool**,** US-unformed stool**,** NSI- No sign of infection**,** FF-Falling of fur

Haematological Profile of Animals

The result on figure 2 represents the mean hematological analysis i.e ESR, PCV, HB, RBC and WBC respectively of the albino rats in the four groups. The result indicated that the mean ESR (Erythrocyte sedimentation rate) in group A was 0.25mm/hr. In group B, the mean ESR for all the animals was 0.00mm/hr, in group C, the mean ESR of animals was 1.00mm/hr. And in group D, the mean ESR of animals was 0.50mm/hr.

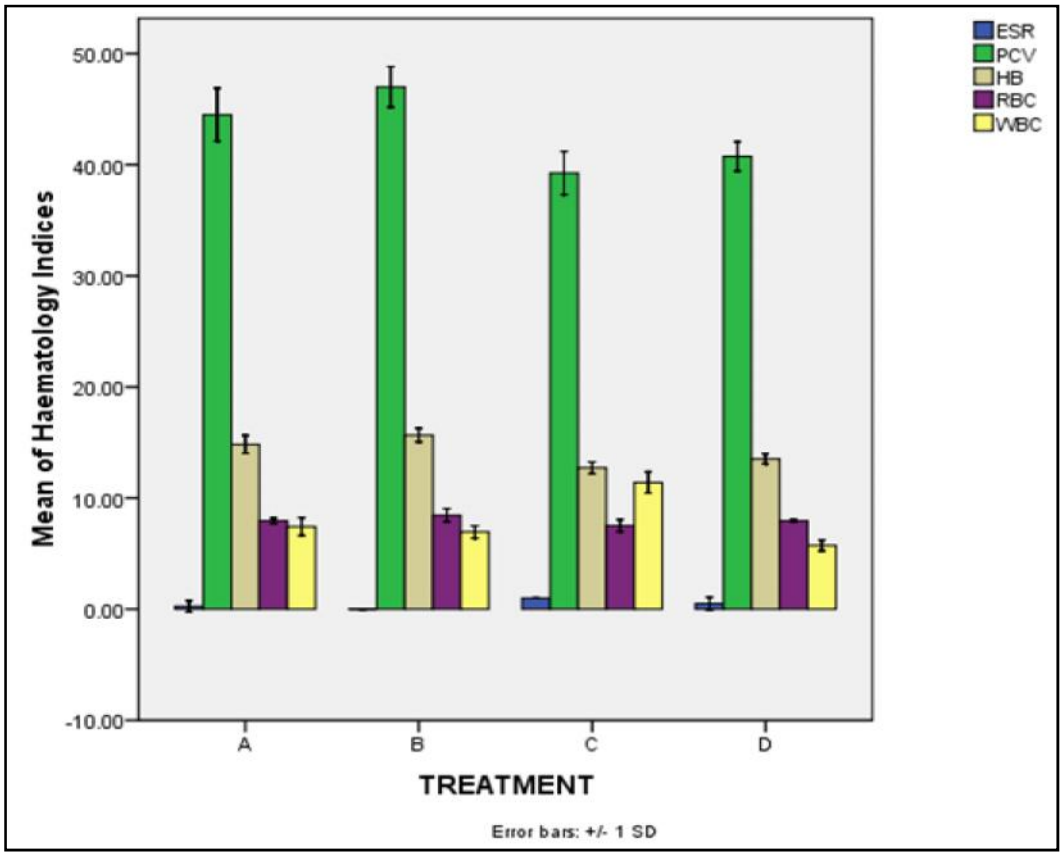

Figure 2: Showing the Mean Haematology of the Albino Rats

KEY: ESR-Erythrocyte sedimentation rate, PCV-Packed cell volume, HB-Haemoglobin, RBC-Red blood cell count, WBC-White blood cell count, A-Infected and treated with olive oil. B-infected and treated with chloramphenicol, C-infected and not treated, D-Not infected (control group)

The result of PCV (Packed cell volume) too indicated that the animals in group B had the highest with mean PCV of 47.00%. This was followed by those of animals in group A, 44.50%. While the mean PCV for animals in group D was 40% and group C had the lowest PCV with mean 39%.

The mean HB (Hemoglobin) count as shown indicated that the animals in group B had the highest count with mean of 15.657g/dl, this was followed by animals in group A 14.825g/dl while animals in group D had mean HB values of 13.525g/dl and those in group C had the least HB with mean of 12.717g/dl.

The mean RBC (Red blood cell) count of the animals indicated that animals in group B had the highest counts of 8.45×10^{12}/L. Animals in group A were next with mean of 7.95×10^{12}/L while group D had 7.95×10^{12}/L also and the lowest RBC count was recorded in group C which had mean of 7.52×10^{12}/L.

The mean WBC (White blood cell) count indicated that animals in group C had the highest WBC count of 11.40×10^{9}/L, those in group A were next with 7.4×10^{9}/L. While the animals in group B followed with 6.95×10^{9}/L and the least WBC count was recorded in group D which had the least mean value of 5.72×10^{9}/L.

WBC Differential Count

Figure 2 represent the mean value of WBC differential count (lymphocytes, neutrophils, monocytes, eosinophils, basophils). Animals in group A had the highest lymphocytes count with mean of 76.75%. Animals in group B were next with mean of 74.75%.This was followed by group D with mean of 71.25% while group C animals had the lowest count with mean of 69.00%.

Neutrophil count as shown indicated that animals in group C had the highest count with mean value of 24.75%, animals in group B and D were next as they had the mean of 22.00%. Animals in group A however had the least with mean of 19.25%.

The monocytes count indicated that animals in group D had the highest count with mean of 5.75%, while group C followed with mean of 5.00%. Animals in group A had mean value of 3.25% and group B had the least with mean of 2.75%.

The eosinophil count indicated that group D had the highest with mean of 1.00% followed by groups A and C with mean values of o.75%, while the least was recorded in group B which had 0.500%.

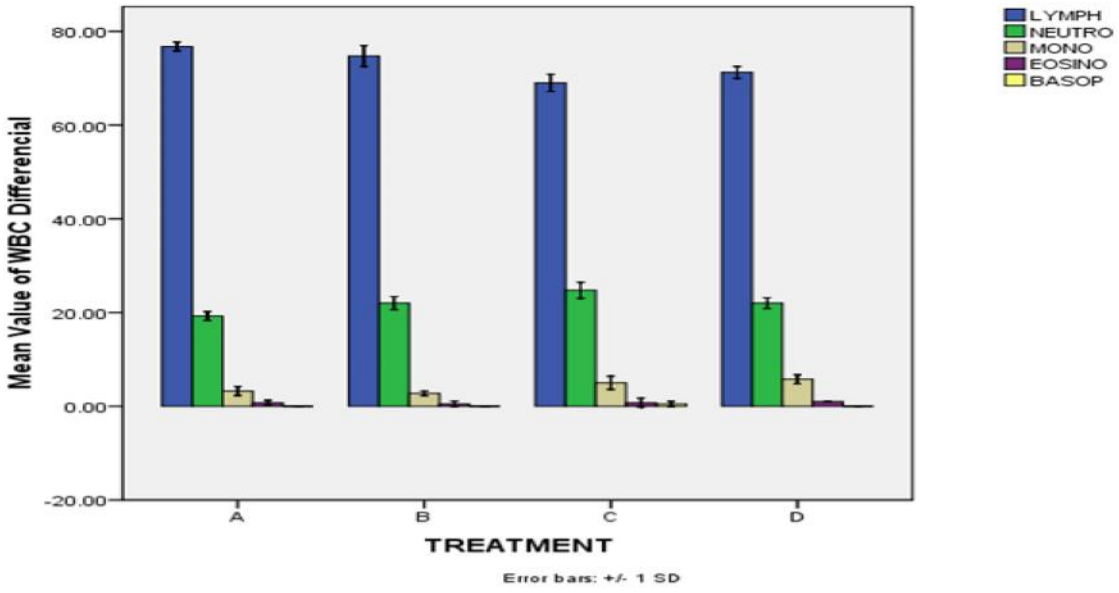

Figure 3: showing the mean WBC differential of the albino rats.

KEY: Lymp-Lymphocytes, Neutr-Neutrophils, Mono-Monocytes, Eosino-Eosinophils, Baso-Basophils., A-Infected and treated with olive oil, B-Infected and treated with chloramphenicol, C-Infected and not treated, D-Not infected and not treated (Control group)

Result of Isolation from Faeces (Stool)

The isolation from the stool samples of albino rats after the treatment period from the four groups indicated that groups A-C (infected) shed *Escherichia coli* in their stool while those in group D shed no *E. coli* in their stool. Group C shed more *E.coli* (i.e had more bacterial count) in their stool compared to those of groups A and B. Group A too shed a little more compared to group B. These isolates when subcultured and then grown on Eosine methylene blue agar (EMB) appeared as shinning metallic sheen.

Result of isolation from urine

The isolation from the urine samples of the animals showed no visible growth on Nutrient agar.

Result of isolation from olive oil

The isolation from Goya extra virgin olive oil showed two colonies that grew from the pour-plate of NA, non was found on the PDA plate. A biochemical test was however carried out on these colonies and the organism was identified as *Staphylococcus epidermidis.*

DISCUSSION

The animals were monitored for weight loss, the weight of animals in all the groups as expected increased during the period of acclimatization but the animals demonstrated a drop in their weights after day 0-1 of infection and this could be ascribed to the fact that the animals were deprived of food and water and subsequently lost their appetite for food immediately after *Escherichia coli* 0157:H7 infection. After *E. coli* 0157:H7 inoculation and commencement of treatment, the animals in groups A and B started to return to their pre-infection weights after day 2 of post-infection (13^{th}day) of the experiment. This may be due to the varying effectiveness of the two treatment plans. Animals in group C had slight weight gain but they never gained back their pre-infection weights till the experiment ended and this may be because they were not placed on any treatment plan resulting in significant weight losses influenced by the bacterial challenge. While the body weight of the control group (D) was a constant trend (i.e increasing) during the experiment. This result is in agreement with the work of Momoh *et al*., (2012) in which rats not subjected to any infection increased constantly in weight.

Olive oil is generally available commercially and it is used without prescription for treatments of various ailments. The present study have shown that feeding animals with diet supplemented with extra virgin olive oil (EOO) does not affect the animals adversely as indicated that the animals infected and treated with extra virgin olive oil (EOO) had high HB, PCV and RBC counts. This can thus be attributed to the fact that olive oil contains vitamins E and K both of which are important for cardiovascular health and helps improve blood circulation (Esterbauer *et al*., 1991). They also contain minerals such as iron, calcium, potassium which support the cardiovascular system and RBC's ability to carry oxygen to all cells in the body and this suggests that the oxygen carrying capacity of the blood is not reduced (Mayne, 1999).The WBC counts of animals in this group (A) was reduced compared to those in group C (infected and not treated animals) but higher compared to the control group (D).The possible reduction suggests that extra virgin olive oil is associated with a greater reduction in gene activity related to inflammation due to infections (Kremer, 1996). Their WBC differential counts indicated that they presented the high counts for lymphocytes, eosinophils, lower counts for neutrophils and monocytes while basophils were insignificant.
Animals in group B (infected and treated with chloramphenicol) did not really exhibit serious signs of the illness. This is because chloramphenicol is a broad spectrum antibiotics that exerts a bacteriostatic action in fighting infections as it inhibits bacterial enzyme peptidyl transferase (an enzyme that catalyze peptide bond formation), thereby preventing the growth

of the polypeptide chain during protein synthesis. The animals remained healthy and they had higher PCV, HB and RBC counts but however had lower WBC counts compared to those of group C (infected and not treated animals) but higher than those of group D (not infected and not treated animals. The result of the WBC differential of this group also indicated that they had higher white blood cell differential counts for lymphocytes and neutrophils while counts for monocytes and eosinophils were low and basophils counts were insignificant.

Generally animals in group C (infected and not treated) had lower PCV, HB, and RBC compared to the other three groups (A, B and D) indicating anemia. This is because acute inflammation from most pathogenic organisms result in haemolysis which is generally manifested in lower HB, PCV and RBC levels (Kumarnsat *et al.,* 2006). They however showed increase in the total circulating leucocytes (WBC counts) as expected, this is because leucocytes are known to increase sharply when infection occurs as the first-line of defense. Their result of WBC differential indicated significant increase in the percentage of circulating neutrophils compared to the other groups (A, B and D). This according to Cheesebrough (2004) shows the onset of bacterial infection thereby, facilitating the removal of bacterial and antibody-coated antigen. Their monocyte counts were higher as compared to the other groups (A and B) but lower than those of group D (control group). This is because monocytes present antigens to macrophages and dentric cells which migrate quickly to sites of infection in the tissues and ingest invading bacteria. There was however a drop in the lymphocyte counts of animals in group C and low lymphocyte counts indicate that the body has low resistance to infection. This is in line with the work of Aboderin and Oyetayo (2006) on haematological studies carried out on rats fed with different probiotic isolated from fermented corn slurry which showed that neutrophils are usually higher during active infection while lymphocytes are lower. The counts for eosinophils were higher while they presented insignificant values for basophils. While the result of haematology of the control animals in group D (not infected and not treated) were normal with moderate PCV, HB, RBC and low WBC counts.

The result of isolation from stool samples (faeces) from the four (4) groups (A-D) revealed that all the infected animals (groups A-C) shed *E.coli* when bacterial counts were estimated (via pour-plating of serially diluted samples and subsequent subculturing on Eosine methylene blue agar). The levels of viable bacteria in faeces were not significantly different in *E.coli* 0157:H7 infected and treated groups (A and B) but there was significant difference when compared to those of *E. coli* 0157:H7 infected and untreated group (C) as counts were higher. Also moderate signs/symptoms were observed in treated animals (groups A and B) compared to the infected and untreated animals (C) in which stool consistency was more apparent with severe signs and symptoms (watery diarrhea). This result demonstrated the effectiveness of the two treatment plans although in varying degrees. While the result of isolation from urine of experimental animals showed no visible growth on Nutrient agar. Though the Goya extra virgin olive oil (EOO) used for this study is expected to be pure (without contaminant), the isolation of *Staphylococcus epidermidis*, a gram positive, non motile bacteria from it may be due to the fact that the organism is a permanent and ubiquitous colonizer of human skin and the probability of contamination may have resulted during the handling process in the laboratory (Uckay *et al*., 2009).

CONCLUSION AND RECOMMENDATION

Nutritional status is generally recognized as an essential factor involved in the modulation of immune response which may be a determinant of development of clinical effects derived from malnutrition process (Chandra, 1996). Lipid though of varying composition, when administered may alter different functions of the immune system through different mechanisms. Numerous reports have suggested that extra virgin olive oil (EOO), an important dietary lipid can act as biological mediators in conditions of inflammation and help to resolve inflammatory diseases, may be effective in the treatment of some autoimmune diseases and

immune regulation (Bowers 2006). Subsequently, this experimental study has demonstrated that a diet containing extra virgin olive oil (EOO) exerted a protective effect in experimentally induced diarrhoea in the albino rats used for the experiment (i.e EOO has antibacterial property) and the animals were not adversely affected as indicated in their haematological profile. However, there is need to conduct further test on its antiviral and antifungal property (EOO) as well as its dosage and duration of use.

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