Original Research Article

THERAPEUTIC EFFECT OF GOYA EXTRA VIRGIN OLIVE OIL IN ALBINO RAT OROGASTRICALLLY DOSED WITH *SALMONELLA TYPHI*

Abstract

The therapeutic properties of Goya extra virgin olive oil on albino rats orogastrically dosed with *Salmonella typhi*were accessed in this study. Both the *in- vivo* and *in- vitro* assays were used in assessing the antimicrobial activity of the Goya extra virgin olive oil. Thirteen microorganisms made up of eight bacteria and five fungi were used in the *in- vitro* bioassay. Comparison of the antimicrobial efficacy of olive oil and commercial antibiotics revealed ofloxacin and gentamicin to be generally more potent against the test organisms than the olive oil. The *in- vivo* bioassay were carried out using twenty albino rats randomly assigned into four study groups of five rats per group. The groups orogastricallly dosed with *Salmonella typhi* revealed that the animals showsdepressed activity and weakness characterised by slow movement, anorexia, falling fur and rough hair coat, light soft faeces, ocular discharge and loss of weight. Following treatment with antibiotic (Ofloxacin) all the characteristic symptoms of the disease decreased and with time, the animals gained more appetite for food and water as revealed by the weight gained by animals after treatment (an average of 7g) which was found to be higher than those gained by the animals treated with Goya extra virgin olive oil (an average of 2g) revealing that the antibiotics is more effective in treating the disease. The control group had a fairly constant colonial count per gram (10^6cfu/g) of animal faeces which ranges from 1.52 ± 0.01 to 1.70 ± 0.01. There was a sharp decrease in the bacterial colony count of the faeces of the animals treated with antibiotic from 3.22 ± 0.06to 1.70 ± 0.01compare to those fed with olive oil which decreased from 3.00 ± 0.00to 2.9 ± 0.03 indicating that the elimination rate of the bacteria in the host is higher with antibiotics than with olive oil. Olive oil is a natural antimicrobial non- toxic immune modulator, it is an amazing health building supplement which stimulate the immune system to fight against infection.

Keywords

Antimicrobial, bioassay,*Salmonella typhi,* Goya extra virgin olive oil, orogastrically

INTRODUCTION

Olive oil is pale yellow to greenish oil with a very characteristic tastegotten from olives pulp by parting the liquids from solids. In the antiqueage, olive oil was applied for lighting, in the arrangement of food, also as an anointing oil for both ritual and aesthetictenacities. Olive oil is veryesculentand edible and a suitable basis of vitamin E. Traditionally, the products of *Olea europaea* active components and clinical applications of Olive Oil have been used as aphrodisiacs, emollients, laxatives, nutritives, sedatives, and tonics. Specific conditions traditionally treated include colic, alopecia, paralysis, rheumatic

pain, sciatica, and hypertension (Gilani *et al*., 2005). Olive oil's characteristic aroma, taste, colour, nutritive properties, and stability distinguish it from other edible vegetable oils. The encouragingeffect of olive oil on health comprises an enhancement in blood lipid profile by reducing the bad LDL-cholesterol (Low Density Lipoprotein) level while considerably raising the level of good HDL-cholesterol (High density Lipoprotein) in the blood stream (EFSA, 2011). Consumption of olive oil reduces coronary hearth diseases, diabetes, certain cancer risks such as breast, prostate and colon cancers, certain malignant tumours (endometrium, digestive tract, skin tumours) and some other chronic diseases (Perez-Jiminez*et al.,* 2005). Olive oil alsouse its biological advantagesmostlyby constituent antioxidants (Cicerale et al., 2012). Though, olive oil composition is multifaceted, the main groups of compounds believed to contribute to its observed health benefits include oleic acid, squalene, sterols (as β-sitosterol), polyphenols (tyrosol, hydroxytyrosol, oleuropein and many others), tocopherols, terpenoids, and traces of other constituents (Covas *et al.,* 2006; Owen *et al.,* 2000) in which they are found to constrain oxidative stress.

Antimicrobialactivity of hydroxytyrosol, Phenolic, tyrosol, and oleuropeinagainst several strains of bacteria implicated inintestinal and respiratory infections have been proven *in vitro* studies. Phenolic compounds have been shown to inhibit the growth of *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (Fabiani*et al.,* 1998; Paster *et al.,* 1988). *Oleuropein* has also been demonstated to inhibit sporulation of *Bacillus cereus* (Tassou, 1991). Hydroxytyrosol is an active antioxidant that has been the subject many investigationreports has shown several biological properties, particularly anti-inflammatory, antifungal, antiviral and antibacterial activities. Hydroxytyrosol resulted effective against clinical human pathogenic strains of *Haemophilus influenzae*, *Moraxella catarrhalis*, *Salmonella typhi*, *Vibrio parahaemolyticus* and *S.aureus* (Bisignano *et al*., 1999). Increasing resistance to antibiotics, wide-spread use of immune-suppressing drugs and a rise in bacterial infections emphasize the necessity to find and develop new antimicrobial agents.

Many investigations have shown the consumption of olive oil to reduce coronary heart diseases, diabetes, certain cancer risks such as breast, prostate and colon cancers, certain malignant tumours (endometrium, digestive tract, skin tumours) and some other chronic diseases. However, there has not been enough literature on the therapeutic properties of consuming the oil. This study is therefore focused on providing relevant information on the therapeutic properties of Goya extra virgin olive oil

MATERIALS AND METHODS

Microorganism used in the bioassay

The microbial isolates used for this project were obtained from the laboratory of microbiology department, federal university of technology Akure, Ondo state, Nigeria. The gram positive bacteria used include *Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus),* and *Streptococcus pyogenes (S. pyogenes)* while the gram negative bacteria used include *Klebsiella pneumonia (K. pneumonia), Salmonella typhimurium (S. typhimurium), Shigella dysenteriae (S. dysenteriae), Escherichia coli (E. coli), and Pseudomonas*

aeruginosa (P. aeruginosa). The fungi used were *Aspergillus niger (A. niger), Penicillium chrysogenum (P. chrysogenum), Aspergillus fumigatus (A. fumigatus), Aspergillus flavus (A. flavus)* and*Neurospora crassa (A. crassa)*

Animals' treatment and diet

Handling and treatment of animal's protocol were strictly adhered to the laid down rules in the ethical guide. Albino rats were used to determine the therapeutic properties of Goya extra virgin olive oil in which sixteen adult albino rats weighing between 60 and 120 g were obtained from Iwo osun State, Nigeria and used for the study. The animals were transported to the department of microbiology, Faculty of science, Federal university of technology Akure, Akure Nigeria. They were randomly assigned into four study groups of five rats per group. They were housed in woody cages with wire screen top and kept under adequate ventilation and the environmental temperature. The animals were maintained on a commercial rat chow with tap water and food (finisher)provided to the rats and following acclimatisation and infection, a group of the infected rat were treated with Goya extra virgin olive oil (a product of Goya Andalucía manufactured in Espana Spain which was purchase from Nao supermaket Oja Oba market Akure, Ondo State, Nigeria.)

Antimicrobial sensitivity testing

Dilution method for antimicrobial sensitivity test

The antimicrobial test was done according to the method of Olorunfemi *et al.*(2006). Aseptically with the aid of a sterile pipette, 1ml of 24hours old peptone broth culture of the test organisms were added to 20ml sterile molten NA and PDA which had already cooled to 45^0C. This was well mixed and poured into previously sterilised petri dishes and allowed to set. With the aid of a sterile 6mm cork borer, 4 well were bored into the agar.Each antibiotic were prepared to the concentration on conventional antibiotic sensitivity disk, the antibiotic used include nalidixic, nitrofuratoin, cotrimoxazol, amoxicillin, tetracycline, augumentin, ofloxacin, and gentamicin for bacteria and ketoconazoleand nystatin for fungi. About 0.1 ml of each antibiotic and 1 drops (0.2ml) of the olive oil were introduced into the wells. The plates were incubated at 37^0C for 24 hours for NA, while PDA was incubated at 25^0C for 72hours

Preparation of inoculant for infectivity of the animal

Aseptically with the aid of a sterile inoculating loop, a pure strain of the test organism was picked from a preserved slant culture and inoculated into a sterile nutrient broth solution (nutrient agar solution whose gelling factor had been decanted out), the broth culture was shaked thoroughly to ensure even distribution of the organism in the broth. The broth culture was then incubated at 37^0C for 24hours after which the broth culture was centrifuged in a centrifuge so as to harvest the pure decanted cells. The pure cells were further washed by adding sterile water to the sediments and re- centrifuge for 3 more times. The resultant residue cell was obtained by decantation and was transferred into a sterile specimen bottle

which was filled up to the 10ml mark with sterile water for infectivity. 1ml of the prepared organism was ingested orally into the rat with the aid of a syringe.

Infectivity assay and treatment

Following acclimatisation for one week, Animals in groups B to D were infected with the prepared inoculant of *Salmonella typh*i and the rats were left without food for 24hours so as to enhance infectivity. While those in group A were left uninfected (control) and given normal feed. After three days of infection, Group B were treated with olive oil in which 1ml of the olive oil was ingested orally into the rat daily, group C were treated with antibiotics (ofloxacin) in which 200mg of the antibiotic was dissolve in 10 ml of sterile water and 1ml of the dissolve drug ingested orally into the rat daily, and group A and D while left untreated with neither olive oil nor antibiotics.

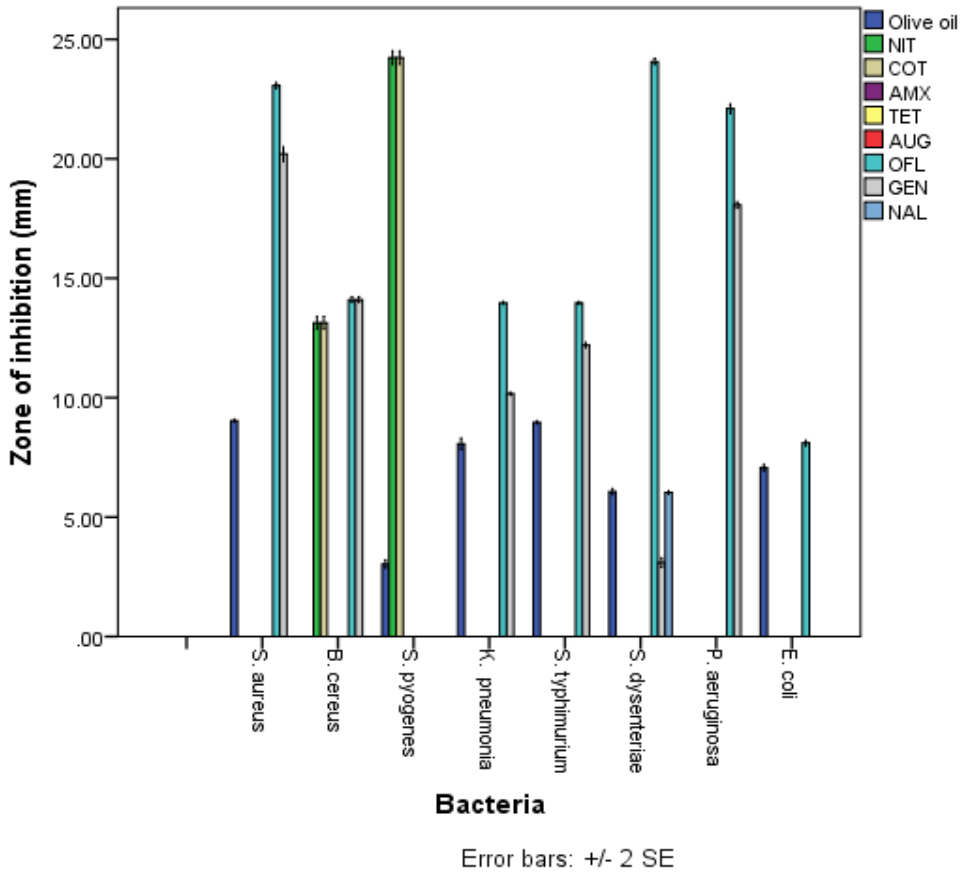
Isolation of bacteria from animal faeces

Bacterial isolation from the faeces of the animals was done before and after treatment.

RESULT:

Antimicrobial sensitivity assay

The result of the antibiotic sensitivity assay on gram positive and gram negative bacteria were shown in table 1. Olive oil was observed not to have any inhibitory activities on one of the Gram positive organism (*Bacillus cereus)* while organism such as *Streptococcus pyogenes and staphylococcus aureus* were a little bit sensitiveand the inhibitory activities of *Streptococcus pyogenes* is much lesser to nitrofurantoin being the only antibiotic that inhibits the organism. Antibiotics such as amoxicillin, cotrimoxazol, and nalidixic were also found to have no inhibitory effect on any of the gram positive organism. Generally, on Gram negative bacteria ofloxacin showed higher antimicrobial activities than the olive oil. Nalidixic and contrimoxazol had approximately the same effect as that of the olive oil on *Shigella dysenteriae* while on the other hand, olive oil was found to have higher inhibitory activities on *Shigella dysenteriae* than gentamicin. The result also showed that all of the gram negative test organisms were resistant to amoxicillin and nitrofuratoin making the olive oil to be more effective than the antibiotics as shown in figure 1. *Penicillium chrysogenum* which was resistant to ketoconazole was more sensitive to olive oil than the remaining test organism as shown in figure 2.



Where NAL= Nalidixic, NIT= Nitrofuratoin, COT= Cotrimoxazol AMX= Amoxicillin, TET= Tetracycline, AUG= Augumentin OFL= Oflaxacin, and GEN= Gentamicin

Figure 1: A bar chart comparing the antimicrobial sensitivity of olive oil with antibiotics for some selected bacteria

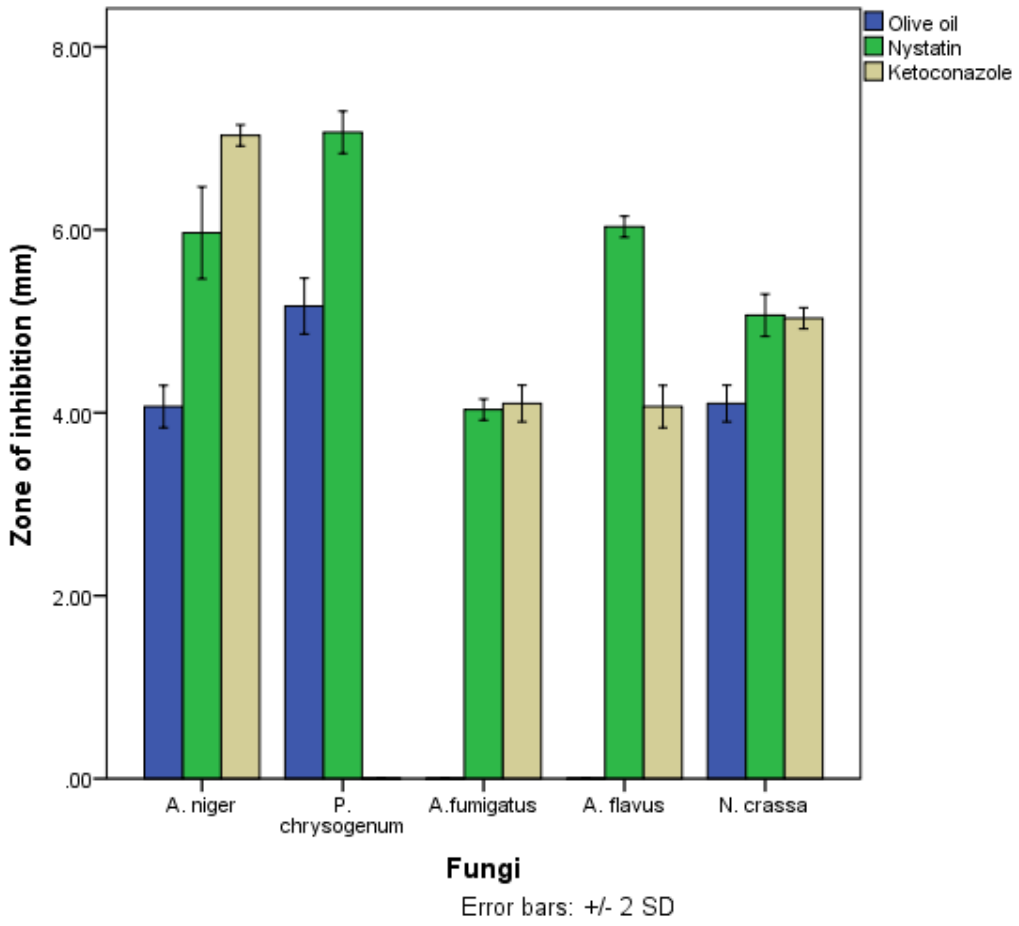


Figure 2: A bar chart comparing the antimicrobial sensitivity of olive oil with antimycotic drugs for some selected fungi

Bacteria isolated from faeces of the animals

Group A (control group) had a fairly constant colonial count per gram ($\times 10^6$cfu/g) which ranges from 1.52 ± 0.01 to 1.70 ± 0.01. It was also noted that group C had the highest colonial count of 1.92 ± 0.02 before infection while group D had the highest colonial count of 3.52 ± 0.02 after infection. Generally, the group treated with antibiotic had a lower bacterial colonial count than those treated with olive oil as can be seen on table 1.

Table 1: **Bacterial colonial count of faeces of the animals before and after treatment (x 10^6 cfu/g)**

GROUP	Before infection	During infection of group B,C, and D	After treatment Of group B and C
Group A	1.52 ± 0.01^a	1.70 ± 0.01^a	1.61 ± 0.01^a
Group B	1.81 ± 0.06^c	3.00 ± 0.00^b	2.91 ± 0.03^c
Group C	1.92 ± 0.02^d	3.22 ± 0.06^d	2.13 ± 0.07^b
Group D	1.72 ± 0.05^b	3.12 ± 0.06^c	3.52 ± 0.02^d

Key

Group A: Control group

Group B: Group infected and fed with olive oil

Group C: Group infected and treated with antibiotics

Group D: Group infected and left untreated

Weight of the wistar rat

The weight of the wistar rat is shown in table 2.

Table 2: Percentage change in body weight of infected Wistar albino rats after treatment with fermented sample

Groups	**Initial weight (g)**	**After two weeks of acclimatisation (g)**	**After infection had set in (g)**	**After treatment (g)**	**% increase in weight**
A	104.20 ± 0.05^a	113.80 ± 0.28^b	118.0 ± 0.38^c	128.33 ± 0.81^d	8.75%
B	91.20 ± 0.16^c	100.61 ± 0.29^d	85.23 ± 0.23^a	87.04 ± 0.29^b	2.12%
C	89.77 ± 0.18^c	97.71 ± 0.39^d	82.26 ± 0.23^a	87.42 ± 0.50^b	6.27%
D	72.29 ± 0.22^c	79.33 ± 0.01^d	72.62 ± 0.28^b	60.12 ± 0.55^a	-0.02%

Data are represented as mean ± standard error (n=3) with the same superscript down the column are not significantly different ($p<0.05$).

Key:

Group A: Control group

Group B: Group infected and fed with olive oil

Group C: Group infected and treated with antibiotics

Group D: Group infected and left untreated

DISCUSSION

Olive oil was found to have higher inhibitory activities on *Shigella dysenteriae* than gentamicin.Olive oil had approximately the same effect as Nalidixic and contrimoxazol on *Shigella dysenteriae*. The major groups of compounds thought to contribute to the observed inhibitory effect of olive oil include the polyphenols (tyrosol, hydroxytyrosol, oleuropein and many others. Hydroxytyrosol, Phenolic, tyrosol, and oleuropein have been shown by some researchers to have mild antimicrobialactivity against several strains of bacteria implicated inintestinal and respiratory infections. Paster *et al* (1998) have shown Phenolic compounds to inhibit the growth of *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus.*Tassou and Nychas (1994) have shown Oleuropein to inhibit the growth of *Staphylococcus aureus.* Hydroxytyrosol is a powerful antioxidant that has been the subject of many research studies and has shown several biological properties, particularly anti-inflammatory, antifungal, antiviral and antibacterial activities. Bisignano *et al* (1999*)* have shown Hydroxytyrosol to effectively inhibit clinical human pathogenic strains of *Haemophilus influenzae*, *Moraxella catarrhalis*, *Salmonella typhi*, *Vibrio parahaemolyticus* and *S.aureus*. *Bacillus cereus and pseudomonas aeruginosa* were found to be resistant to olive oil with no zone of inhibition meaning that the organisms were able to resist many of the bioactive components of the olive oil using one or more mechanism which conform with the work of walker (2009) who showed that some organisms are resistant to olive oil. All gram positive organisms were found to be resistant to amoxicillin, cotrimoxazol, and nalidixic. Also all gram negative test organisms were resistant to amoxicillin and nitrofuratoin with no zone of inhibition making the olive oil to be more effective than the antibiotics. Resistance to antimicrobial agent could be as a result of bacterial been able to produce enzymes which inactivate or modify antibiotics or pathogens been able to change in their bacterial cell membrane thus preventing the uptake of an antimicrobial agents (Cheesbrough, 2004). Increasing resistance to antibiotics, wide-spread use of immune-suppressing drugs and a rise in bacterial infections emphasize the necessity to find and develop new antimicrobial agents (Buttler *et al., 1996*).

Aspergillus fumigatus and Aspergillus flavus were resistant to olive oil while *Penicillium chrysogenum* which was resistant to ketoconazole was more sensitive to olive oil than the remaining test organism. This mild antifungal properties of olive oil observed in this studies agrees with the work of walker (1996) who showed olive extract to be a natural antifungal agent since they have the potential to destroy many kinds of fungus, or their subdivision of yeasts such as candida. He shows the following as some of their known actions: interference with the production of amino acids within fungal cells needed for their survival, and the stimulation of phagocytosis (engulfing of microbial cells by immune cell). People with compromised immune systems such as those undergoing aggressive

chemotherapy treatments, organ or bone marrow transplant and receiving immunosuppressant drugs or those with immune deficiencies such as AIDS are particularly vulnerable to some fungal infections and olive oil can be a valuable preventative natural antifungal supplement to be taking during some of these treatments (Shoba and Thomas, 2001). Olive oil used in conjunction with a suitable diet including cultured vegetables and high quality probiotics, has proved to be a very successful *candida albicans* natural treatment (Shoba and Thomas, 2001).

In-vitro assay have shown *Salmonella typhimurum* to be more sensitive to olive oil than the remaining test organism and ofloxacin to be more active (showing a higher zone of inhibition) against *salmonella typhimurum* than the remaining test antibiotics which justified the reason while *salmonella typhimurum* and ofloxacin were used *in vivo* as the test organism and antibiotic respectively. Signs and symptoms of infection on animals infected with bacteria (*salmonella typhi*) include depressed activity and weakness characterised by slow movement, lack of appetite for food and water or anorexia, falling fur and rough hair coat, light soft faeces, ocular discharge and loss of weight and this agrees with the work of Holmes, (1984) who showed the above symptoms as signs and symptoms of salmonellosis in a rat infected with salmonella. Following treatment of group B with antibiotic (Ofloxacin) all the characteristic symptoms of the disease decrease and with time the animal gain more appetite for food and water as revealed by the weight gained by animals after treatment (an average of 7g) which was found to be higher than those gained by the animals treated with Goya extra virgin olive oil (an average of 2g) revealing that the antibiotics is more effective in treating the disease. A total decrease of about 15g of weight was noted with the untreated animals after the treatment period and without antibiotic therapy, weakness and weight loss may persist for weeks and animal may die within 1 to 2 weeks (Baker *et al*., 1979). An average of about 10g of weight was gained with the control animals suggesting the fact that the rate of weight gain is higher with the control than with any of the infected group.

The control group had a fairly constant colonial count due to the fact that the animals are not infected. The group left untreated had the highest colonial count after infection. Generally, the group treated with antibiotic had a lower density of the bacterial population than those treated with olive oil indicating that the elimination rates of the infecting bacterial is higher with antibiotics than with Goya extra virgin olive oil. That is, the microbe contributing to the illness are being killed and an increase in energy after taking olive oil is associated to the fact that the microbial load in the body has been reduced which is in conformity with the work of walker (1996) who revealed that animal gain more energy after consuming olive oil

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