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

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Abstract

The therapeutic properties of Goya extra virgin olive oil in 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 Goya extra virgin olive oil. Thirteen microorganisms including 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, gentamicin as well as the olive oil to be potent against the test organisms. 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 showed depressed activity and weakness such as slow movement, anorexia, falling fur and rough hair coat, light soft faeces, ocular discharge and loss of weight**.** Following separate treatments of the rats with antibiotic (Ofloxacin) as well as Olive oil, all the characteristic symptoms of the disease decreased with time. The treated animals gained more appetite for food and water as evidenced by an increase in their weight. The average weights gained by the rats treated with Goya extra virgin olive oil were found to be higher than the weights of the untreated animals. Thus, revealing that Goya extra virgin olive oil has an antibacterial effect as does, antibiotics in the treatment of S. Typhi induced disease. The control group had a fairly constant colonial count per gram (10^6cfu/g) of animal faeces which ranged 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 ± 0.06 to 1.70 ± 0.01 when compared to those fed with olive oil which decreased from 3.00 ± 0.00 to 2.9 ± 0.03. This confirms that the elimination rate of the bacteria in the host is as a result of the antibacterial activity of the olive oil and the antibiotics respectively. Olive oil is a natural antimicrobial and a non- toxic immune modulator, it is an amazing health building supplement which stimulates the immune system to fight against infection. Hence, results from this study have justified the use of olive oil as a natural antimicrobial and a non- toxic immune modulator (since the total phenol content present in Goya extra virgin olive oil was shown to be 14.90). Phenols are the major groups of compounds revealed to contribute to the observed inhibitory effect of olive oil. Also, the result of the mineral analysis of the Goya extra virgin olive oil showed Goya extra virgin olive oil not to have any traces of lead. Presence of lead may lead to poisoning and can cause a number of adverse human health effect. Hence, Olive oil has a significant non- toxic health building supplements which has a therapeutic effect on Salmonella Typhi induced infection.

Keywords

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

INTRODUCTION

Olive oil is a pale yellow to greenish oil with a very characteristic taste gotten from olives pulp by parting the liquids from solids. In the antique age, olive oil was applied for lighting, in the arrangement of food, also as an anointing oil for both ritual and aesthetic tenacities. Olive oil is very esculent, edible, and a suitable source of vitamin E. Traditionally, the products of *Olea europaea* active components and clinical applications of Olive Oil have been used as aphrodisiacs, emollients, laxatives, nutritive, 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 encouraging effect 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 also uses its biological advantages mostly by 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.

Antimicrobial activity of hydroxytyrosol, Phenolic, tyrosol, and oleuropein against several strains of bacteria implicated in intestinal and respiratory infections have been proven in *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 demonstrated to inhibit sporulation of *Bacillus cereus* (Tassou, 1991). Hydroxytyrosol is an active antioxidant that has been the subject many investigation reports 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 and certain cancer risks such as breast, prostate and colon cancers. Also, olive oil has been investigated to reduce 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 oil. This study is therefore focused on providing relevant information on the therapeutic properties of Goya extra virgin olive oil.

MATERIALS AND METHODS

Microorganisms 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

The protocols involved in the handling and treatment of animals were strictly adhered to, according 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 was treated with Goya extra virgin olive oil (a product of Goya Andalucía manufactured in Espana Spain which was purchase from Nao supermarket Oja Oba market Akure, Ondo State, Nigeria.)

Antimicrobial sensitivity assay using the agar well diffusion method

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, four wells were bored into the agar in three different Petri dishes, and each of the antibiotics used was prepared to the concentration of conventional antibiotic sensitivity disk. The antibiotics used include nalidixic, nitrofurantoin, cotrimoxazole, amoxicillin, tetracycline, augumentin, ofloxacin, and gentamicin. While ketoconazole and nystatin were the antimycotic drugs used. About 0.1 ml each of the antibiotics, the antimycotic drugs and a drop (0.2ml) of the olive oil were introduced into the wells respectively. The plates were incubated at 37^0C for 24 hours for NA plates, while for the PDA plates were incubated at 25^0C for 72hours.

Preparation of the inoculant for infectivity of the animals

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 mixed by shaking thoroughly to ensure an even distribution of the organisms 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 three more times. The resultant residue cells were obtained by decantation and were transferred into a sterile specimen bottle which was filled up to the 10ml mark with sterile water for infectivity. With the aid of a syringe, One (1) ml of the prepared organisms was administered orally into the gastrointestinal tract of the rat.

Determination of the infective dose for Salmonella Typhi in Wistar Albino rats

Twenty adult Wistar albino rats grouped into five groups (four in each group) were used in determining the infective dosage for *S. typhi* following the method described by (Komolafe *et al*., 2013).

Infectivity assay and treatment

Following acclimatisation for one week, Animals in groups B to D were infected with the prepared inoculant of Salmonella Typhi (3.05×105 cfu/ml) 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 was treated with olive oil. This was done by administering 1ml of the olive oil orally to the rats daily. The group C rats were treated with antibiotics (ofloxacin) by dissolving 200mg of the antibiotic in 10 ml of sterile water whereby, about 1ml of the dissolved drug was administered orally to the rats daily. The rats in group A and D where left untreated with neither olive oil nor antibiotics.

Isolation of bacteria from animal faeces

Isolations of bacteria from the faeces of the animals were done before and after treatment according to the method described by (Aribisala *et al*., 2018)

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS software version 17.0, SPSS Inc., Chicago, IL, USA). The experiment was conducted using a completely randomized design, and data obtained were analyzed by one-way analysis of variance (ANOVA). Means were compared by Duncan’s new multiple range tests and considered statistically significant when $P \leq 0.05$.

RESULT:

Antimicrobial sensitivity assay

The result of the antibiotic sensitivity assay on gram positive and gram negative bacteria are shown in figure 1. Olive oil was observed not to have any inhibitory activities on one of the Gram positive organisms (*Bacillus cereus)* while organisms such as *Streptococcus pyogenes and Staphylococcus aureus* were a little bit sensitive. The inhibitory activities of *Streptococcus pyogenes* were much lesser to nitrofurantoin (the only antibiotic that inhibited the organism). Antibiotics such as amoxicillin, cotrimoxazole and nalidixic acid were also found to have no inhibitory effect on any of the gram positive organism. Generally, ofloxacin showed higher antimicrobial activities on Gram negative bacteria than the other antibiotics. Nalidixic acid and cotrimoxazole had approximately the same effect as that of olive oil on *Shigella dysenteriae.* Also, olive oil was found to have higher inhibitory activity on *Shigella dysenteriae* than gentamicin. The result also showed that all of the gram negative test organisms were resistant to amoxicillin and nitrofurantoin making the olive oil to be more effective than the antibiotics as shown in figure 1. Olive oil was found to have a significant inhibitory effect (with a zone of inhibition of 5.00mm) on *P. chrysogenum* when compared to Ketoconazole which has no inhibitory effect on the same microorganism. *Penicillium chrysogenum* which was resistant to ketoconazole was more sensitive to olive oil than the other test organism as shown in figure 2.

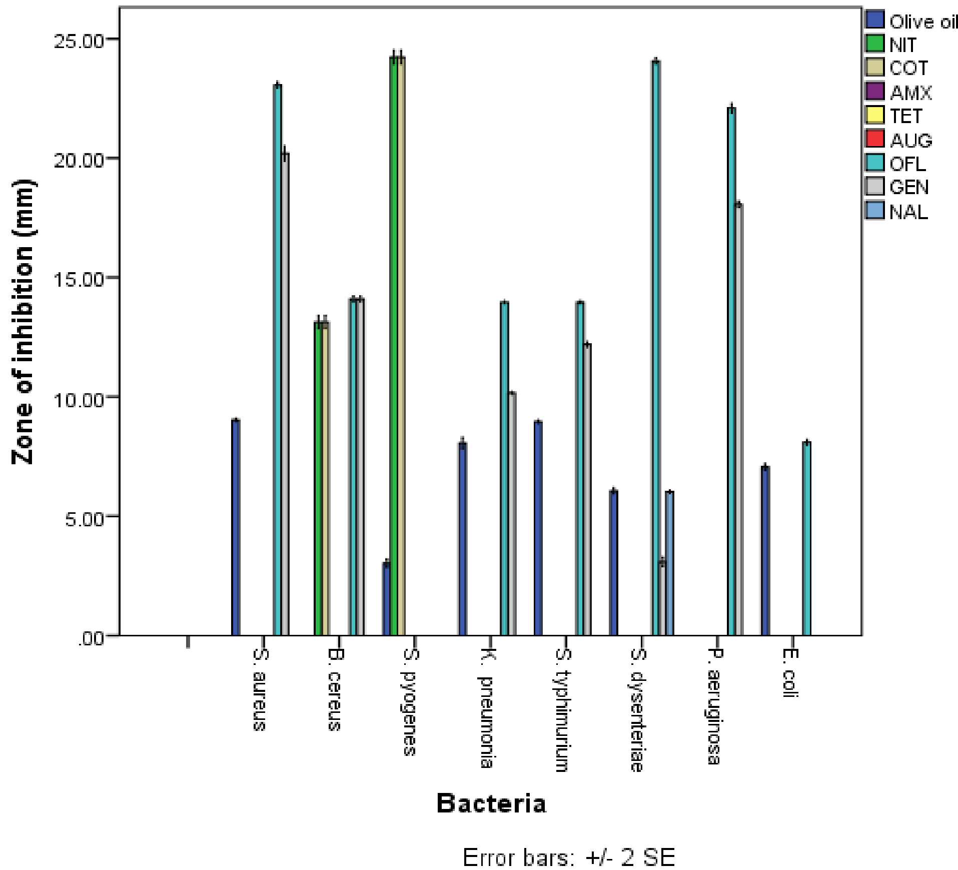

Figure 1: A bar chart comparing the antimicrobial sensitivity of olive oil with antibiotics for some selected bacteria.

NAL= Nalidixic, NIT= Nitrofurantoin, COT= Cotrimoxazole AMX= Amoxicillin, TET= Tetracycline, AUG= Augumentin OFL= Oflaxacin, and GEN= Gentamicin

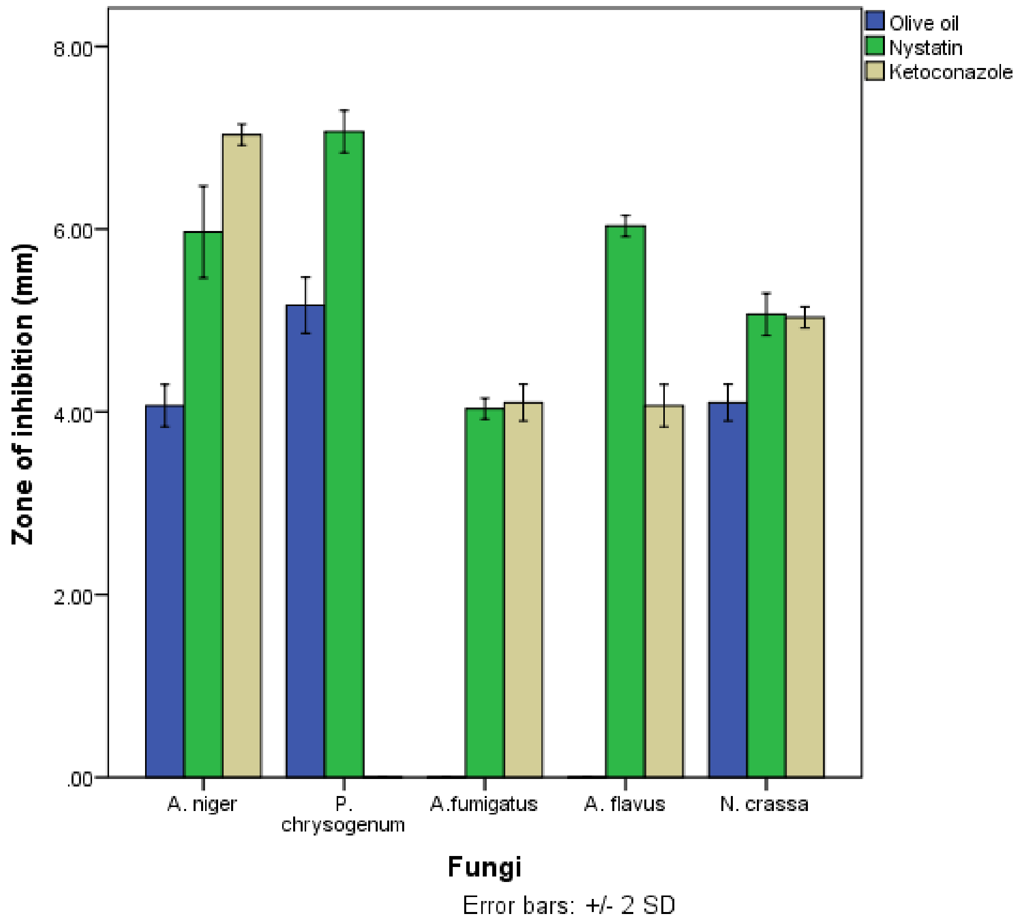

Figure 2: A bar chart comparing the antimicrobial sensitivity of olive oil with antimycotic drugs for some selected fungi

Bacteria isolated from faeces of the animals

Group A (control group) had a fairly constant colonial count per gram 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 (group C) had an anti-bacterial effect as well as those treated with Olive oil (group B). This is evidenced by the lower bacterial colonial counts when compared with the Group D untreated rats (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 rats

The weights of the wistar rats are shown in table 2. It was noted that the Olive oil as well as the antibiotic treated groups had an increased weight of 87.04±0.29^{b} and 87.42±0.50^{b} grams respectively after treatment. This shows that Olive oil as well as the antibiotic, had an antibacterial effect on the treated rats when compared to the lesser weights (60.12±0.55^{a} grams) of those left untreated in group D

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

Bioactive constituents in olive oil

The result of the physicochemical analysis of the Goya extra virgin olive oil was shown in table 3 in which the free fatty acid present was 1.36mg/g, saponification value was found to be 2.72mg/g, acid value to be 2.72mg/g and the specific gravity was 0.91g/cm^{3}. The result of the mineral analysis of the Goya extra virgin olive oil was shown in table 4 and it was noted that no traces of lead was present in the oil. The total phenol content present in Goya extra virgin olive oil was 14.90± 0.189. Result expressed as Mean ± Standard deviation.

Table 3: Physicochemical properties of the olive oil

PARAMETER	RESULT
SPECIFIC GRAVITY(g/cm3)	0.91± 0.02
ACID VALUE (mg/g)	2.72± 0.033
FREE FATTY ACIDS (mg/g)	1.36± 0.09
PEROXIDE VALUE (mg/kg)	18.80±0.01
SAPONIFICATION VALUE	173.77± 0.31

Result Expressed as: - Mean ± Standard deviation

Table 4: Minerals properties of the olive oil

PARAMETER	RESULT
LEAD (Pb)	BDL
IRON (Fe)	0.02
ZINC (Zn)	0.43

BDL = below detection limit

DISCUSSION

This study focused on the therapeutic effect of Goya extra virgin olive oil in albino rats orogastricallly dosed with Salmonella Typhi. The presence of antibacterial bioactive components in Goya extra virgin olive oil corroborates with the findings of (Aribisala *et al*., 2017). The researchers identified phenol derivatives as the major groups of compounds thought to contribute to the observed inhibitory effect of olive oil.

Olive oil was found to have higher inhibitory activities on *Shigella dysenteriae* than gentamicin. Olive oil had approximately the same effect as Nalidixic and cotrimoxazole 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 antimicrobial activity against several strains of bacteria implicated in intestinal 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. This suggests that the organisms were able to resist many of the bioactive components of the olive oil using one or more mechanism. This conforms with the work of walker (2009) who showed that some organisms are resistant to olive oil. Gentamycin had no effect on *E. coli* which according to (Johnson *et al*., 1994) suggests that, this resistant isolate contains a gene encoding the enzyme 3-N-aminoglycoside acetyltransferase type IV (AAC[3]IV) that mediates resistance to gentamycin. All gram positive organisms were found to be resistant to amoxicillin, cotrimoxazole, and nalidixic. Also all gram negative test organisms were resistant to amoxicillin and nitrofurantoin with no zone of inhibition making the olive oil to be more effective than the antibiotics. Resistance to the antimicrobial agents could be as a result of the bacteria been able to produce enzymes which inactivate or modify the antibiotics. It may also be as a result of a pathogen been able to change/alter their cell membranes, thus preventing the uptake of the 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 study agrees with the work of walker (1996) who showed olive extract to be a natural antimycotic drugs 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, anorexia, falling fur and rough hair coat, light soft faeces, ocular discharge and loss of weight. This agrees with the work of Holmes, (1984) who revealed the above symptoms as those of salmonellosis in a rat infected with salmonella. Following treatment of group B rats with an antibiotic (Ofloxacin), all the characteristic symptoms of the disease decreased and with time, the animal gained more appetite for food and water as evidenced by an increase in weight (an average of 7g). The weights gained by the rats treated with Ofloxacin were found to be higher than the weights gained by the animals treated with Goya extra virgin olive oil (an average of 2g). Thus, revealing that Goya extra virgin olive oil has an antibacterial effect as does, antibiotics in the treatment of S. Typhi induced disease. A total decrease of about 15g in weight was noted with the untreated animals after the treatment period. There was a persistence of symptoms such as weakness and weight loss among the untreated rats. This conforms to the report of (Baker *et al*., 1979) who stated that; weakness and weight loss may persist for weeks and animal may die within 1 to 2 weeks. An average of about 10g of weight was gained with the control animals suggesting the fact that the rate of weight gain is higher among the control group of animals 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. The group treated with antibiotic (Ofloxacin) had a lower density of the bacterial population than those treated with olive oil indicating that; although, both have antibacterial properties but the elimination rates of the pathogens were higher when treated with antibiotics than with Goya extra virgin olive oil. That is, the microbes contributing to the illness are being eliminated and increased energy in the rats after taking the olive oil suggests that, the microbial load of the pathogens in the rats had been reduced. This is in conformity with the work of walker (1996) who revealed that animals gain more energy after consuming olive oil.

CONCLUSION

Although the use of antibiotics has generally been tempered to avoid antibiotic resistance, the development of resistance by microorganisms is inevitable hence, the results of this study is significant for the development of Goya extra virgin olive oil to be utilized as an augment for the current antibiotics therapy that is becoming less efficacious against antibiotics resistant Salmonella Typhi. This study showed that Goya extra virgin Olive oil contains bioactive components which displayed protective measures against the pathogenic symptoms caused by Salmonella Typhi in induced mice. The antimicrobial action of the oil justifies its use as a therapeutic agent. Likewise, its effect against bacteria and fungi makes it a broad spectrum in activity.

Ethical Approval: The protocols involved in the handling and treatment of animals were strictly adhered to, according to the laid down rules in the ethical guide

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