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Evaluation of the Effect of Co-Administration of Acalypha Wilkensiana and Allium Sativum on Kurga (Makia-Kia) Infected Young Albino Wistarss Rats

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Abstract:- *Kurga* or *makia-kia* is a disease of neonates and infants, common to Plateau, Nassarawa and Kaduna states of Nigeria, and said to be more pathological in male children than female. Although the disease is well known among the local population, unfortunately, medical professionals have often denied the existence of such a disease due to the absence of scientific data to back up such a claim. The disease is characterized by dermatological irregularities such as skin patches, often around the nose and face, skin ulcers and desquamation, greenish –yellowish mucoid stools, abdominal discomforts, anal ulceration, constipation and neonatal hemorrhoids. Most parents whose infants were affected by the disease often claim that hospital prescriptions were ineffective, thereby resolving to alternative medicines, prominent amongst which is *Acalypha wilkensiana* or *Allium sativum*. Thus, the aim of this study is the 'Evaluation of the effect of co- administration of *Acalypha wilkensiana* and *Allium sativum* on *kurga* or *makia –kia* disease, provide some scientific basis to ascertain the existence of the disease and to isolate the causative pathogen. The study employed a combination of qualitative and experimental research design. Questionaires were used to sample out the opinions or experiences of local women within some of the affected areas in order to establish a theory pertaining the diseases. Young albino wistar rats, weighing between 250 to 800g were divided into five groups, with each group, made up of five young rats. Causative pathogens isolated from stool samples collected from infants who have shown 85% of the disease symptoms were used to induce the disease into four of the affected groups , designated as infected untreated group (IR) , infected treated group (ITR1), which were administered 100mg/kg bw of the extracts mixture, ITR-2 which were administered only *Acalypha wilkensiana* aqueous mixture at a dose of 100mg/kg bw , and finally, ITR-3 , which were administered aqueous extract of *Allium sativum* at 100mg/kg bw. Thereafter, stool and blood samples were collected for analysis and documentation. The qualitative approach revealed 89% of the study respondents, affirming the existence of the disease, with only 11% denying. 78% of the women admitted to have had children who were affected by the disease, most of whom were between 0 and 6 months old at the time. 94% of such parents belief that hospital prescriptions were ineffective against the disease, while 76% admitted to taking their affected wards to herbal homes. Ninety percent (90%) of the samples obtained from infants who have presented with at least 80% of the disease symptoms revealed E.*coli* isolates. It is thus safe to establish that kurga disease does exist and is caused by E.*coli*, and could be managed effectively by *Acalypha wilkensiana* and *Allium sativum*, except in cases of co-infection with other pathogens with no registered susceptibility.

Keywords:- *Kurga, Makia-Kia.*

I. INTRODUCTION

Kurga or *makia-kia* is a disease of neonates common to Plateau, Nassarawa, Borno and Kaduna states of Nigeria. Although the disease is well known among the local population, unfortunately, medical professionals have often denied the existence of such a disease, possibly due to the absence of scientific data to back up such a claim.

The disease is characterized by dermatological irregularities such as skin patches (often around the nose and face) ,skin ulcers or desquamation and dryness. Other symptoms include abdominal discomforts, usually preceding bowel movements, constipation and greenish –yellowish,

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mucoid stool. Advanced form of the disease according to the locals, is often associated with anal ulceration and neonatal haemorrhoids.(sourced from local women). The disease is said to be more pathogenic in male children than female.

Most parents whose neonates or infants were affected by the disease often claim that hospital prescriptions have been ineffective, thereby making them resolve to alternative medicines, prominent among which are *A.wilkensiana* or *Allium sativum* solution. *Acalypha wilkensiana* is a member of the spurge family (Euphorbiaceae) belonging to the genius *Acalypha* and is commonly called copper leaf , Joseph's coat and fire dragon.(Chollom 2010).

Acalypha wilkensiana is a popular outdoor plant native to Fiji and nearby islands in the South Pacific, but has spread to most parts of world , especially the tropics of Africa, America and Asia.(Gotep et al 2016).

The number of diseases reportedly managed by the use of *A.wilkensiana* has made scientists seek the biochemical basis of its medicinal importance. Prominent among such scientists are Gotep, et al.,(2016) who carried out antimicrobial screening using ethanol extracts of *A.wilkensiana* . According to their study , the extract was potent against a number of microbial agents such as *staphyloccoccus aureus, yersinia enterocolitica, E.choli, salmonella typhi,pseudomonas aeroginosa and klebsienna aerogenes*; a great number of which have been accused of causing GIT diseases ,skin diseases e.t.c. Similarly, Ikewuchi and Ikewuchi (2010) examined the antidiabetic activity of *A.wilkensiana* on blood sugar and cholesterol levels of rat models. According to their findings, aqueous extracts of *A.wilkensian* had a lowering effect on the above mentioned parameters; thereby making it an alternative of choice in the management of cardiovascular diseases.

Ogbuchi, et al., (2014) studied the protective effect of *A.wilkensiana* on biomarkers of oxidative stress in liver homogenates, using 70% methanol , extract which was administered intraperitoneally in a dose of 50mg/kg and 100mg/kg of the extract for a period of 14days. A significant decrease ($p<0.05$) in malondialdehyde levels in the liver was registered ; whereas , a significant increase in the activity of superoxide dismutase and catalase in both 50mg/kg and 100mg/kg administered groups compared to control.

Medicinal plant such as Garlic (*allium sativum*) contain certain substances which possess chemotherapeutic agents beneficial to man animals alike. According to Mushin et al (2007), E.*coli* and S .*aureus* showed a great deal of sensitivity to garlic water extract, just as Cavalito and Bailey (1944) registered themselves as the first scientists to discover that the andtibacterial tendency of garlic was due to its *allicin* content.

Cellini et al in their 1996 findings demonstrated that garlic extracts possess a broad spectrum of antimicrobial activity against certain Gram –ve and Gram +ve bacteria such as E.*choli, Salmonella , Staphylococcus Streptococcus ,Klebsiella , Proteus, Bacillus* and *Clostridium.* Similarly, Esmone et al (2010) also showed the potency *Allium sativum* against C. *albicans* species , E. *choli* and S. *aureus* .

II. LITERATURE REVIEW

Any plant in which one or more of its components contain substances that could deliver therapeutic benefits and or can be used in the production of useful drugs according to Burkil in 2004 is called a medicinal plant. According to Ward and Hetzel, in 1999, all plants existed for human benefits. Similarly, Gundiza in 1985 documented that the medicinal characteristics of plants is dependent on the presence of certain phytochemicals such as alkaloids, anthraquinones, cardiac glycosides, tannins and polyphenols.(Gundiza, M, 1985)

Since time immemorial, phytotherapy has been used in the management of various diseases. Herbal medicine is practiced by about 75- 80% of the world's population, mostly in the developing countries. *Acalypha wilkensiana*, commonly called Irish petticoat is a plant native to the South Pacific islands and belong to *Euphorbiaceae* family.(Ikewuchi et al, 2010)

The value of medicinal plants to the health of communities is indispensable; however, plants used in traditional settings for the sole purpose of medical therapy are still inadequately studied. *Acalypha wilkensiana* is used singly or as a major constituent of herbal mixtures in traditional society, for the management of a number of diseases. (Chollom et al 2010).

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Garlic possesses a great deal of therapeutic properties including antimicrobial, anti- neoplastic, anti-caridiovascular, immune –stimulatory and hypo-glycaemic activities. Based on the works of Haris (2001), and Singh (2008), garlic (allium sativum) shows both antimicrobial and pharmacological effects. To hit the nail on the head, Hughes and Lawson in 1991 established that, the whole antibiotic activity possessed by garlic is lost if its thiosulfate contents is removed. (Hughes et al 1991)

Allium sativum belongs to the family *Liliaceae* and the genius *Allium*. It has bulb-like root consisting of several bulbils (cloves) and the bulb is milkish in colour. It possesses a strong, powerful, arid and penetrating odour / smell, which according to Dulta in 1998 is used as a condiment in the preparation of meat and fish delicacies.

Common places where *allium sativum* is found are India, China, Southern Europe, North America and Northern Nigeria.

In 2006, Stoll and Seebeck reported that Allicin content of allium sativum is the main reason for its pharmacological characteristic. Worthy of note is the fact that garlic has never been found to contain a substance considered poisonous to life. (Stoll et al 2006).

The scientific works of Gomaa and Hashish (2003) documented that allium sativum extract inhibited the growth of *shigella dysenteriae*, *E.coli* , *staph. Aureus*, *salmonella* typhi ,e.t.c. Galli (1985) also reported that garlic extract inhibited gram- positive and gram – negative bacteria such as *Bacilli* and *Clostridia*, *Yeast* and *moulds.*

Okoye, who compared the bacterial effect of garlic on *S.aureus* and *E.coli* with commercially prepared nalidixic acid (0xoid) and tetracycline (oxoid) on *S.aureus*, tetracycline gave a zone of inhibition of 20mm, less than 22mm of garlic extract inhibition zone.(Okoye, 2010).

A Palestinian scientist by name Zakaria in 2003 revealed that the peak inhibitory effect of Allium sativum on S. Faecalis is obtained at a concentration of 50mg/ml.

Groppo et al., in 2002 established that a marked sensitivity was observed on oral *streptoccus* to garlic extract; similarly, garlic extract mouth wash reduced the total counts of salivary bacterial and mutant streptococci.

Rubin Dasgupta et al (2012) reported in India that 0.1ml of 10% (w/v) of garlic extract, poured into different wells, provided an inhibitory variation of between 19.68 – 20.75mm with mean of 20.22mm diameter of inhibition zone against S.*aureus*.

A. The Concept of Kurga or Makia-Kia Disease of Neonates and Infants

Kurga or *makia- kia* is not an English name but a combination of Berom and Alago dialects of Plateau and Nassarawa states of Nigeria respectively. The name is used to refer to the local disease common among infants between ages 0 to 2years old. There has been little or no scientific work yet on this local infection, and so no English or scientific name has been attached to the disease yet.(sourced from local women).

B. Cause of Kurga or Makia-Kia Disease of Infants and Neonates

The actual cause of *kurga* or *makia-kia* disease of infants and neonates has not yet been established. Therefore, one of the specific objectives of this scientific work is to determine the cause of the disease and to proffer effective management or cure for it.

C. Symptoms of Kurga or Makia- Kia Disease of Infants and Neonates

Before now, there has been no scientific documentation of the symptoms of *kurga* or *makia –kia* disease of infants and neonates ; however , the observed symptoms usually reported by parents, and noted in the course of these research includes : dermatological irregularities like skin patches (often around the nose and face) scaling, skin ulcers, skin desquamation ,skin lacerations and dryness. Other symptoms include abdominal discomforts, usually preceding bowel movements, constipation and greenish –yellowish, mucoid stool. Advanced form of the disease is usually marked by anal ulceration and neonatal haemorrhoids, accompanied by mild bloating and anaemia.(sourced from local women).

D. Care and Management of Kurga or Makia-Kia Disease of Infants and Neonates

The management and care of infants with *kurga* or *makia-kia* disease has been largely traditional therapy, as most of the locals in the affected region belief that it is not a disease that could be managed conventionally. This therapy involves boiling the leaves of *A. Wilkensiana* or any other plants considered effective in that locality, for several minutes. Next, the coloured extract is given orally to both the mother and child once daily. In some occasions, the child's bathing water is also mixed with the extract to treat dermatological irregularities. The daily therapy is concluded at night before retirement with warm drips of the extract to the child's anus to stimulate stool excretion. This is based on the belief that frequent bowel movements amount to quicker elimination of the disease. (sourced from local women).

E. Medicinal Use of Acalypha Wilkensiana

The aqueous extract of *Acalypha wilkensiana* showed different activity against *Staphylococcus aureus* , *Yersinia* , *Enterocholitica*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeroginosa* and *Klebsiella aerogenes*; all of which have been implicated in diseases of the gastrointestinal tract,(Gotep et al,2016). Acalypha wilkensiana has also been reportedly used in the management of diabetes mellitus and cardiovascular diseases. And has been shown to reduce cholesterol levels in serum.(Ikewuchi et al,2010).

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F. Medicinal Use of Allium Sativum

Initially, the vast majority of people are drawn to *Allium sativum* due to its unique test in food , however, a good number of scientists have demonstrated its abundant potential in the management of certain bacterial infections. *Allium sativum* extracts successfully inhibited the growth of *Escherisa coli*,*Staphyloccoccus aureus* and *Salmonella typhi* (Gomaa et al, 2003). Similarly,*Allium sativum* has been used worldwide for centuries by different communities to fight different pathogens , and its antibacterial activity is believed to be inherent as a result of its allicin content.(Janah et al,2015).

G. Management of Kurga or Makia-Kia Infection in Clinics and Hospitals

One of the major factors militating against the proper diagnosis and finding of effective therapy for Kurga or Makia-kia disease is the outright denial of its existence even in the face of glaring symptoms by medical professionals. Medical professionals would often belittle parent's genuine worry any time their infants begin to show symptoms of the disease by calling it a problem of poor hygiene which could be solved by improving cleanliness. They would later prescribe very good paediatric antibiotics formulations which are often ineffective. At other times, they would encourage the parents to breast feed the child more with the believe that increased breastfeeding would translate to higher maternal immunity which could create a higher capacity for immune response against the 'so called condition'. For these reasons, there is no specific formal management of the disease in hospitals or clinics. However, two logical questions could be raised from the above approach of professionals towards this public health challenge: One, 'Why prescribe antibiotics to manage a non existing disease?. And secondly, 'Why the desire to stimulate immune response against nothing?

III. METHODS

A. Collection of Plant Materials.

Acalypha wilkensiana and *Allium sativum* plants were obtained from a local garden belonging to Ronicon Hotel along Rayfield Road Jos, Plateau state, and from the local abattoir market Jos south, respectively. The plants were identified and authenticated at the Federal School of Forestry, Jos, Plateau state.

B. Preparation of Plant Extracts

Acalypha wilkensiana leaves and *Allium sativum* pulps were collected, air dried at room temperature under the shade for three weeks. They were then pounded to powdery form using local mortar and pestle. 160g of *Acalypha wilkensiana* and 160g of *Allium sativum* were obtained respectively and dissolved in 1000mls . This was boiled for 15 minutes, and then filtered using whatman filter paper No.1 to remove unextractable matter. The filtrate were concentrated in a water bath at a temperature of 60 degrees and stored in air tight containers until needed. The dried samples were then later reconstituted with distilled water to give the required dosage of 100mg/kg and 150mg/kg body weight.

C. *Design*

This study employed a combination of qualitative and experimental research design.As a guide to this research, a pilot toxicity assay using Lorke's method in which three groups of three young rats each were administered different doses of 10, 100 and 1000mg/kg body weight of the mixture in the first phase, and yet a second phase in which the animals were again divided into three groups of three animals each and administered higher doses of 1600, 2900 and 5000mg/ kg body weight respectively. This toxicity test revealed the highest dose that gave no mortality as 100mg/kg body weight and a lowest dose that gave mortality as 1600mg /kg body weight. The mean lethal dose was 532mg/ kg bw.

D. Qualitative Approach

Questionnaires were used to sample out the opinions, feelings or experiences of local women within some of the communities in the affected areas in order to establish a theory pertaining *kurga* or *makia-kia* disease of neonates and infants.

E. Experimental Approach

Inclusion Criteria : Young albino wistar rats, weighing between 250- 800g were used in this research.

Experimental Animals : A minimum of twenty five albino wistar rats were provided and divided into five groups, with each group having at least five rats.

F. Induction of kurga or makia-kia disease of neonates and infants

The selected experimental animals were first screened to rule out the presence of any underlying bacterial pathogens. *Kurga* or *makia-kia* disease was induced into four of the five groups by lacing of the animal feed with pathogens isolated from infected human infants through a two stage culture period, after which their stool samples were observed for symptoms of the disease after a four day period . A secondary culture of the animal faeces was done to re-establish the culprit's presence.

G. Administration of the Extract

The aqueous extract of the mixture of *A.wilkensiana* and *allium sativum* shall be administered through oral route at a dose of 100mg/kg body weight after the secondary culture of the experimental animal faeces confirms the presence of the culprits and not before. This implies that a young rat weighing at least 250g shall receive 25mg/ml , containing 0.16mg and 0.16mg of *A.wilkensiana* and *Allium sativum* respectively . Similarly, one out of the five groups shall be administered 100mg/kg body weight separately with aqueous extracts of *allium sativum* and *acalypha wilkensiana* while one group each shall be administered individual , aqueous extracts of *A .wilkensiana* and *A. Sativum* at a dose of 100mg/kg body weight respectively.

H. Group Designations

Group A: Normal control rats (NC) – non infected rats, fed with normal diet for 7days.

Group B:Infected control rats(IR) –rats were infected but not treated and fed with normal diet for 7 days.

Group C: Infected treated rats (ITR-1) rats were infected with the disease , fed with normal diet and then treated with *Acalypha wilkensiana* and *Allium sativum* extracts mixture for 7days at 100mg/kg body weight.

Group D: Infected treated rats (ITR-2) – these group were also infected with the disease ,fed with normal diet and then treated with the aqueous extract of *acalypha wilkensian* at a 100mg/kg body weight for 7 days .

Group E : Infected treated rats (ITR 3) – these groups were also infected with the disease, then fed with normal diet and treated with aqueous extract of *Allium Sativum* at a 100mg/kg body weight.

I. Feeding of the Animal

The rats were fed with normal diets and in addition, groups C,D and E were treated for 7 days through intragastric tube.

J. Blood Collection

The administration of the extract lasted for 7 days, after which the experimental animals were decapitated and their blood samples collected for analysis. The blood samples were collected into separate test tubes for each of the group: those for clinical biochemistry were collected in plain tubes and allowed to clot for 40 minutes, while that for haematological assays were collected in EDTA bottles, and then prepared accordingly for analysis.

K. Phytochemical Screening of Plant Extract

The phytochemical screening of the powdered aqueous extract of *Acalypha wilkensiana* and *Allium Sativum* were achieved by the use of standard qualitative procedure as laid down by Trease and Evans (1984) and Sofowora, (1993).

L. Test for Alkaloids

Extraction: Ten grams (10g) of sample was taken in a small beaker and a concentrated solution of ammonia was added in a quality sufficient to just moisten it and allowed to stand for 10 minutes after thorough mixing of the contents. Sufficient quantity of mixture of chloroform and ethanol solution (1:1) was added just to soak and suspend the powder. The mixture was allowed to stand for 20 minutes with occasional stirring with a rod. The mixture was filtered through a plug of cotton wool. The solution was washed twice with 2ml of chloroform and the washed sample was combined with the filtrate. The residue was cooled and dissolved in 5ml of chloroform only. The chloroform solution was transferred to a small separating funnel and shaken with 3ml of dilute sulphuric acid. The layers were allowed to separate; the chloroform lower layer drained off and discarded. Three mililitres of chloroform was further added, shaken, drained off and discarded until upper acid layer was colourless. The acid layer was made completely alkaline with strong ammonia solution (tested with indicator paper). The extraction with 3ml of chloroform extracts were retained and evaporated to dryness, the residue was dissolved in 3ml of ethanol and the following test were carried out after neutralizing with dilute sulphuric acid.

Dragendor Reagent Test For Alkaloids To 2.0ml of extract few drops of the reagent were added and observed for orange colouration to indicate the presence of alkaloid (Sofowora: 1993).

M. Test for Flavonoids Extraction

Five grams (5g) of the sample was completely destained with acetone. The residue was extracted in warm water evaporation of the acetone on a water bath. The mixture was filtered and the filtrate was used for the following test.

Lead Acetate Test To 2.0ml of extract, 10% lead acetate solution was added and observed for either cream or light yellow colouration confirmed the presence of flavonoids (Sofowora, 1993).

N. Test for Tannins Extraction

Three grams (3g) of the powdered sample was boiled in 50ml of distilled water for 3 minutes on a hot plate. The mixture was filtered and the resulting filtrate was used to carry out the following test for tannins.

- *Ferric Chloride Test:*

One mililitre of extract was diluted with 4.0ml water (in a ration of 1.4) and few drop of 10% ferric chlororide solution were then added. The solution was then observed for blue or green precipitate or colouration to indicate the presence of tannins (Trease and Evans, 1984).

O. Test for Saponins

Froth Test: To 0.5g of the powdered sample , 10mls of 95% ethanol was added and boiled. The mixture was added 10 10ml of distilled water in a test tube was stoppered and shaken vigorously for about 30 seconds, and then it was allowed to stand for over half an hour. Honey-comb front is indicative of the presence of saponins (Sofowora, 1993).

P. Test for Cardiac Glycosides Extraction:

Zero point five grams of the powdered sample was boiled with 10ml of 95% alcohol for 2 minutes. The resulting mixture was filtered and cooled. The filtrate was diluted with water and three drops of a strong solution of lead sub-acetate was added. This was mixed thoroughly and filtered. The filtrate was divided into two portions. One portion of the filtrate was kept for the test below:

- *Salkowski Test Cardiac Glycosides:*

To 2.0ml of the filtrate, 2.oml of conc. H_2SO_4 was carefully added down the side of the tube while observing for the formation of a layer of interphase of reddish brown colour indicative of cardiac glycoside (Sofowora, 1993).

Q. Test for Terpenes and Steroids Extraction

Five grams of the powdered sample was extracted by maceration with 50ml 95% ethyl alcohol then filtered and the filtrate was evaporated to dryness. The residue was dissolved in 10ml of anhydrous chloroform and the filtered. The filtrate

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was divided into two equal portions and the following test was carried out.

➢ *Liebermann-Burchard Test:*

2.0ml of the extract was mixed with 1.0ml acetic anhydride followed by the addition of 1.ml conc. H_2SO_4 carefully down the side of the test tube while observing for the formation of layer of interphase of reddish brown colour which indicates the presence of terpenes and steroids (Sofowora; 1993).

R. Test For Phenols

To 2.0ml of the extract was added few drops of chloroform and 2ml of $FeC1_3$ a deep bluish green colouration indicates the presence of phenol (Sofowora; 1993).

S. Test for Resins

Zero point five grams of the powdered sample was dissolved in acetic anhydride and 1 drop of concentrated sulphuric acid was added . A purple or violet colour indicate the presence of resins (Sofowora , 1994).

T. Termination Culture

After the conclusion of the treatment period, fresh stool samples were collected and re-inoculated from all the three groups to determine the degree of antimicrobial activity of the extract mixture, invitro.

U. Culture Technique

A total of ten (10) stool samples were collected from infants between ages 0 and 12months , who have presented atleast 80% of the disease symptoms. A primary inoculation of the samples was done on equal number of petri dishes containing already prepared DCA media, ready for use, and then cultured at 37 degree Celsius for a period of 24 hrs. After 24 hrs, the isolates were purified and re-inoculated in a less clustered pattern on a secondary DCA media and re-cultured at 37 degree Celsius for another 24 hrs period.

V. Culture plate reading

Isolates obtained from the two stage process above were read and identified based on the following parameters: colour, size, elevation, edge and consistency which could be soft, mucoid or hard.

W. Organism identification

The identification of the specific microbes down to their species was achieved by subjecting the isolates to a number of biochemical tests which included, triple sugar iron agar test, citrate test, catalase test, urease test, indole test and glucose test.

Catalase test – this is performed to differentiate staphylococci species from streptococci. It tries to determine wether or not an organism is able to split H_20_2 to release oxygen gas.

Indole test –this is a biochemical test carried out to differentiate gram negative bacilli or enterobacteria. This is seen in the organism's ability to split indole from tryptophan.

Coagulase test –this test is carried out to determine if the organism possess the enzyme coagulase.

Urease test –urease agar is a biochemical differential medium that tests the ability of an organism to produce the exoenzyme called urease that hydrolyzes urea to ammonia and carbon dioxide.

Citrate test –this is a biochemical test that assesses the ability of an organism to use citrate as the sole source of carbon and energy.

X. *Preparation of isolated organism for induction of the disease*

The isolated and identified organisms obtained from above were re-inoculated into sterile peptone water (broth), which was then used to lace the feed meant for the experimental animals.

Fig 1 Isolates Prepared in Broth for Induction of the Disease

Fig 2 Culture Isolates from Infant Stool Samples

Y. Experimental parameters and methods

A number of biochemical parameters were tested and tabulated from the test subjects from all the four groups. These parameters include:

➢ *Renal Function Test*

Serum Urea –this shall be determined using the method of Tobacco et al., 1979.

Principle of test: urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by berthelot's reaction.

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Urea + H_20 ----------$2NH_3 + C0_2$

NH_3 + Hypochloride + Phenol ---------- Indophenol (blue compound)

➢ *Procedure:*

	Blank	standard	sample
Sample	--------	--------	0.01ml
STD	----------	0.01ml	---------
Reagent1	---------	0.1ml	0.1ml

Mix and incubate at 37 degree celcius for 10 minutes.

Reagent 2	2.50ml	2.50ml	2.50ml
Reagent 3	2.50ml	2.50ml	2.50ml

➢ *Mix immediately and incubate at 37 degree Celsius for 15 minutes.*

- Read absorbance at 546nm.
- *Serum Uric acid* –this was determined by the method of Fossati et al.,(1980) using Randox commercial test kits.
- *Principle*: uric acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3,5-dichloro -2-hydrobenzensulfonic acid and 4- aminophenazone to form a red –violet quinoneimine compound.
- *Serum electrolytes* –sodium, potassium and chloride were determined by the principle of flame photometry, which states that the intensity of light emitted, is directly proportional to the concentration of the test metal or electrolyte under study.
- *Serum creatinine* – principle: creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration in the sample.
- *Liver Function Test:-* All liver enzymes were determined by Reitman and Frankel method of 1957 using Randox commercial test kits.
- *Aspartate Aminotransferase (AST) Principle*: An amino group is enzymatically transferred by Aspartate amino-transferase present in the sample to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate. AST activity is thereafter measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.
- *Alanine Aminotransferase (ALT) Principle*: An amino group is enzymatically transferred by Aspartate amino-transferase present in the sample to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate. ALT activity is thereafter measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine
- *Serum total protein* – principle: cupric ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a coloured complex.
- *Serum albumin* –principle: The measurement of serum albumin is based on its quantitative binding to the indicator 3,3-5,5 –tetrabromo –m- cresol sulphonephthalein (bromocresol green, BCG). The albumin – BCG- complex absorbs maximally at 578nm , the absorbance being directly proportional to the concentration of albumin in the sample.
- *Total bilirubin* –principle: this is based on colorimetric method based on that described by Jendrassik and Grof (1983). Direct (conjugated) bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotized sulphanilic acid.

Z. Haematological parameters

All haematological factors such as packed cell volume, total white blood count and total erythrocyte shall be determined using the principle of flow cytoflourimetry.

This principle is based on the fluorescence emitted when individual cells are attached to fluorescent chromophores. The flouresence emitted is directly proportional to the number of cells under measurement.

IV. RESULTS

A. Description

The table below shows the different pathogens isolated from ten(10) stool samples of infants who have shown atleast 80% of the disease symptoms , ranging between ages two(2) to twelve (12) months old. *Escherichia coli* was isolated from all the samples and were accompanied by *Klepsiella spp* and *Proteus mirabilis* in samples one (01) and nine (009) respectively. This suggest that *Escherichia coli* is the culprit causing *Kurga* or *Makia-kia* disease.

Table 1 Showing Isolated and Identified Organisms From Cultured Samples.

S no.	Age In Months	Sample Type	Organism Isolated
1	4	STOOL	E.COLI
2	3	STOOL	E.COLI
3	5	STOOL	E.COLI
4	7	STOOL	E.COLI
5	10	STOOL	E.COLI
6	9	STOOL	E.COLI
7	3	STOOL	E.COLI
8	2	STOOL	E.COLI
9	4	STOOL	M./E.COLI
10	12	STOOL	E.COLI

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B. Demographic Characteristics of Study Respondents

Table 2.0 shows the characteristics of the study respondents. A total of 100 people made up of 98 adults females and 2 adult males took part in the study. 95 of the 98 females are married, with 88 of them having more than three children. 52% of the respondents possess national diploma, and 33% having a senior school leaving certificate. These suggest that most of the respondents possess adequate experience and a good literacy level to understand the topic under discuss.

Table 2 Demographics of Study Respondents

Age (in years)	**20-25**	**25-30**	**30-70**		
	0	64	36		
Sex	M	F			
	2	98			
State of origin	Plateau	Others states			
	71	29			
Number of Children	One	Two	Three or more		
	2	10	88		
Highest aced. Qualification	PRM	SSCE	ND	HND	B.SC
	10	33	52	4	1

M =Male F=Female Prm =Primary School Leaving Certificate

SSCE= Senior School Leaving Certificate ND= National Diploma

HND= Higher National Diploma B.Sc = University Degree

A total of 89% responded in the affirmative to knowing about the disease, while only 11 % said otherwise. 78% of the women admitted to have had children who were affected by the disease, most of whom were between 0 and 6 months old, at the time; suggesting a period of weak or growing immunity. Similarly, 76% of such parents took the affected children to the local herbalist instead of a conventional health facility, as 94% of them belief that hospital prescription are ineffective against the disease. This therefore establishes the fact that *kurga or makia –kia* disease truly exist and should be given all the necessary attention possible.

Table 3 Awareness or Knowledge of Kurga or Makia-Kia Disease by Respondents

Question	**Response**		
Do you know about a disease called *kurga* or *makia-kia*	Yes		No
	89		11
Where did you learn of the disease?	Hospital/clinic		From local women
	9		91
Has your child or children ever been affected by the disease?	Yes		No
	78		22
How old was the child when he or she was affected by the disease?	0.6mths	6-10mths	12mths
	78	10	12
Where did you take such a child for treatment?	Hospital/Clinic		Local Herbalist
	24		76
Did you try treating your child or children with, hospital, prescriptions against the disease?	Yes		No
	12		88
Would you recommend a hospital or clinic to a friend, in the event of noticing the disease..?	Yes		No
	22		78
Why not hospital prescriptions?	Because they are ineffective 94		
	Because I belief only in herbal medicines 6		

- Phytochemical Components Of The Various Extracts And Mixture.

Table 4.0 shows the results obtained from the phytochemical screening of *Acalypha wilkensiana* , Allium Sativum and that of the aqueous mixture of the two extracts . Alkaloids, tannins, saponins, cardiac glycosides,phenols and anthraquinones were present, except flavonoids and steroids.

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Table 4 Phytochemical Components of the Different Aqueous Extracts

S/N	Phytochemical	A.Wilkensiana	A.sativum Mixture
1	Tannins	+	- +
2	Phenol	+	- -
3	Saponnin	+	- +
4	Flavonoid	+	+ +
5	Cardiac glycoside	+	+
6	Alkaloid	+	+ +
7	Terpenoids	+	+ +
8	Steroids	-	+ -
9	Anthraquinones	+	- -

C. Liver, Kidney and Haematological Analtyes in the Study Groups

Six liver, six renal and three haematological analytes measured during the seven days study period were profiled and their mean, standard deviation, F- test and P- values were tabulated across the four groups and compared with acceptable reference range after subjecting the experimental animals to the extract.

Total proteins (TP) showed no significant variation ($p>0.05$) across the groups ,except for the sharp fall noticed in infected group treated with 100mg/kg bw (ITR-1) ,which showed significant drop ($p<0.05$) when compared with the infected untreated group(IR). Serum Albumin (ALB) levels showed a significant drop ($p<0.05$) when normal control group (NC) was compared infected untreated group (IR). However, a significant rise ($p<0.05$) was noticed in the ITR-1 group as compared with IR . Similarly, a significant rise ($p<0.05$) in haemolysis is observed as seen in the Total Bilirubin levels when NC is put side by side with IR , and again dropped when compared with ITR-1 and ITR-2.

Table 5 Effect of Different Treatment of the Aqueous Plants Extracts on Some Liver Analytes

GR UP	TP(g/l)	ALB(g/l)	T.BIL(mi.mo/l)
NC	73.28±0.03	38.2 ±0.24	13.10 ±0.5
IR	83.24 ±1.42 ^{x}	33.6 ±0.46 ^{b}	18.54 ±0.42 ^{a}
ITR-1	77.72 ±1.63 ^{x}	40.52 ±0.46 ^{b}	10.34 ± 0.23 ^{b}
ITR-2	69 ± 0.12 ^{b}	36.47 ± 0.20 ^{ac}	11.38 ± 0.04 ^{b}
ITR-3	71.34± 0.12^{X}	37.00±0.02^{X}	12.01± 0.05^{X}

NC= Normal control. IR = Infected untreated Rats. ITR-1 = Infected treated group with extract at 100mg/kg bw,

ITR-2= Infected treated group with *acalypha wilkensiana* extract at 100mg/kg bw

ITR- 3= Infected treated group with *allium sativum* extract at 100mg/kg bw.

Data are Mean ± MSD, n =5

^{a} values are significantly higher when compared with normal control($p<0.05$)

^{b} values are significantly lower when compared with infected group ($p<0.05$)

^{c} values are significantly higher when compared across the groups ($p<0.05$)

^{x} values not significant when compared with all groups ($p>0.05$)

A significant rise ($p<0.05$) in ALP serum levels was observed when we compared NC group with IR ,

signifying an infectious state. However,a significant decline was observed down the group following the

administration of different treatments (ITR-1, ITR-2 and ITR-3). Similarly, a significant decrease ($p<0.05$) was observed after the administration of the mixture of the extract as seen between ITR-1 and IR,

As well as in AST levels , to show a decline in hepatotoxicity.

Table 6 Effect of Different Treatment of the Aqueous Extracts on Liver Enzymes

GROUP	ALP(U/L)	ALT(U/L)	AST(U/L)
NC	63.44 ±0.27	55.54±0.26	36.38 ±0.44
IR	78.06 ±0.13 ^{a}	68.74 ± 0.42 ^{a}	37.40 ± 0.55 ^{a}
ITR-1	70.86±0.09 ^{ab}	50.42 ± 0.26 ^{b}	31.40 ±0.01 ^{b}
ITR-2	65.91 ±0.03 ^{ac}	60.00 ± 0.11 ^{ac}	52.74 ±0.04 ^{ac}
ITR-3	66.18 ± 0.01^{a}	59.00 ± 0.21^{a}	12.01 ± 0.05^{x}

NC= Normal control. IR = Infected untreated Rats. ITR-1 = Infected treated group with extract at 100mg/kg bw,

ITR-2= Infected treated group with *acalypha wilkensiana* extract at 100mg/kg bw.

ITR-3 = Infected treated group with allium sativum extract at 100mg/kg bw.

Data are Mean ± MSD, n =5

^{a} values are significantly higher when compared with normal control($p<0.05$)

^{b} values are significantly lower when compared with infected group ($p<0.05$)

^{c} values are significantly higher when compared across the groups ($p<0.05$)

^{x} values not significant when compared with all groups ($p>0.05$)

The table below showed a significant drop ($p<0.05$) in Na^{+} levels when IR group was compared with the ITR-1 group to depict a healthy homeostatic function , just as metabolic acidosis significantly dropped ($p<0.05$) between IR and ITR-1 as seen in Hco_3^{-} rise.

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Table 7 Effect of Different Treatments of Aqueous Plants Extracts on Electrolytes Levels

GROUP	Na^+(mmol/l)	K^+ (mmol/l)	$HC0_3^-$(mmol/l)	Cl^- (mmol/l)
NC	142.18 ± 0.13	5.94 ± 0.10	18.08 ± 0.13	106.50 ± 0.5
IR	146.18 ± 0.18[a]	6.85 ± 0.09[a]	17.40 ± 0.42[a]	110.52 ± 0.48[a]
ITR-1	137.92 ± 0.54[b]	5.84 ± 0.25	20.38 ± 0.35[b]	100.30 ± 0.27[b]
ITR-2	149.72 ± 0.07[ac]	5.00 ± 0.01	19.10 ± 0.20[x]	108.44 ± 0.20[a]
ITR-3	148.11 ± 0.01[a]	5.12 ± 0.41[x]	99.18 ± 0.11[b]	103. 45 ± 0.01[b]

NC= Normal control. IR = Infected untreated Rats. ITR-1 = Infected treated group with extract at 100mg/kg bw,

ITR-1= Infected treated group with extract mixture at 100mg/kg bw.

[a] values are significantly higher when compared with normal control($p<0.05$)

[b] values are significantly lower when compared with infected group ($p<0.05$)

[c] values are significantly higher when compared across the groups ($p<0.05$)

[d] values not significant when compared with all groups($p>0.05$)

The table below showed a significant rise ($p<0.05$) in serum Urea levels when NC group is compared with ITR-1, while a highly significant drop ($p<0.05$) in creatinine levels was observed between IR and ITR-1 to show a healthy creatinine clearance.

Table 8 Effect of Different Treatments of Aqueous Plant Extracts on Renal Analytes

GROUP	Urea (µmol/l)	Creatinine(µmol/l)	Uric Acid(µmol/l)
NC	5.40 ± 0.10	81.65 ± 0.11	123 ± 0.11
IR	6.52 ± 0.29[a]	101.02 ± 0.45[a]	129± 0.21[a]
ITR-1	6.26 ± 0.15	78.65 ± 0.21[ab]	125 ± 0.21[ab]
ITR-2	5.60 ± 0.01	79.10 ± 0.37[bx]	120 ± 0.31[b]
ITR-3	6.00 ± 0.01	78.91 ± 0.12[X]	121 ± 0.03[b]

NC= Normal control. IR = Infected untreated Rats. ITR-1 = Infected treated group with extract mixture at 100mg/kg bw,

ITR-2= Infected treated group with *acalypha wilkensiana* extract at 100mg/kg bw.

ITR-3 = Infected treated group with *allium sativum* extract at 100mg/kg bw.

Data are Mean ± MSD, n=5

[a] values are significantly higher when compared with normal control($p<0.05$)

[b] values are significantly lower when compared with infected group ($p<0.05$)

[c] values are significantly higher when compared across the groups ($p<0.05$)

[x] values not significant when compared with all groups ($p>0.05$)

Table 9 shows a good haematopoetic effect of *Acalypha wilkensiana* as seen in the significant rise in PCV and RBC levels when infected untreated group (IR) is compared with ITR-2.

Table 9 Effect of Aqueous Plant Extract on Haematological Analytes

GROUP	PCV(%)	RBC($X10^{12}$/L)	WBC($X10^3$/mm^3)
NC	33.18 ± 0.25	4.12 ± 0.04	3.34 ± 46
IR	33.96 ± 0.04[x]	4.18 ± 0.11[a]	3.78 ± 0.0[a]
ITR-1	37.48 ± 0.30[x]	4.74 ± 0.06[ab]	3.48 ± 0.07[ab]
ITR-2	38.11 ± 0.01[a]	5.00 ± 0.14[c]	3.08 ± 0.00[x]
ITR-3	37.00 ± 0.81[a]	5.12 ± 0.71[a]	3.44 ± 0.14[x]

NC= Normal control. IR = Infected untreated Rats. ITR-1 = Infected treated group with extract mixture at 100mg/kg bw.

ITR-2= Infected treated group with *acalypha wilkensiana* extract at 100mg/kg bw.

ITR-3= Infected treated group with *allium sativum* extract at 100mg/kg bw.

Data are Mean ± MSD, n=5

[a] values are significanlly higher when compared with normal control($p<0.05$)

[b] values are significantly lower when compared with infected group ($p<0.05$)

[c] values are significantly higher when compared across the group ($p<0.05$)

[x] values not significant when compared with all groups ($p>0.05$)

The table below shows significant mobilization ($p<0.05$) of IgG and IgM at the onset of bacterial proliferation as can be seen when normal control group (NC) was compared with infected group (IR), hence the rapid rise in serum levels of the above mentioned parameters. However, the administration of different treatments of the extracts ,especially co-administration of *Acalypha*

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wilkensiana and *Allium sativum* (ITR-1), caused a significant fall ($p<0.05$) in IgG and IgM serum levels , to depict effective antibacterial potential of the treatment.

Table 10 Effect of Aqueous Plant Extract on Some Immunological Parameters

Group	**IgG(mg/l)**	**IgM (mg/l)**
NC	592.50± 0.11	87.50± 0.24
IR	838.88± 1.01[a]	124.05± 0.41[a]
ITR-1	628.85± 1.42[ab]	92.88± 0.44[ab]
ITR-2	669.04± 0.05[ab]	112.00± 0.60[a]
ITR-3	670.01± 0.13[a]	118.00 ± 0.18[a]

NC= Normal control. IR = Infected untreated Rats. ITR-1 = Infected treated group with extract mixture at 100mg/kg bw,
ITR-2= Infected treated group with *acalypha wilkensiana* extract at 100mg/kg bw.
ITR-3 = Infected treated group with *allium sativum* extract at 100mg/kg bw.
Data are Mean ± MSD, n=5

[a] values are significantly higher when compared with normal control($p<0.05$)
[b] values are significantly lower when compared with infected group ($p<0.05$)
[c] values are significantly higher when compared across the groups ($p<0.05$)
[x] values not significant when compared with all groups ($p>0.05$)

D. Comparative Anti-Microbial Effect of the Individual Constituents of the Mixture on Isolates

The anti-microbial effect of the *A.wilkensiana on E.coli* (Figure 3.0) showed morderate zone of inhibition . Although the anti-microbial effect of *A.wilkensiana on E.coli* colonies is not adequate for an effective therapeutic measure , it is however, far better than that seen with *Allium sativum* (figure 4.0) , which has a far less significant zone of inhibition.

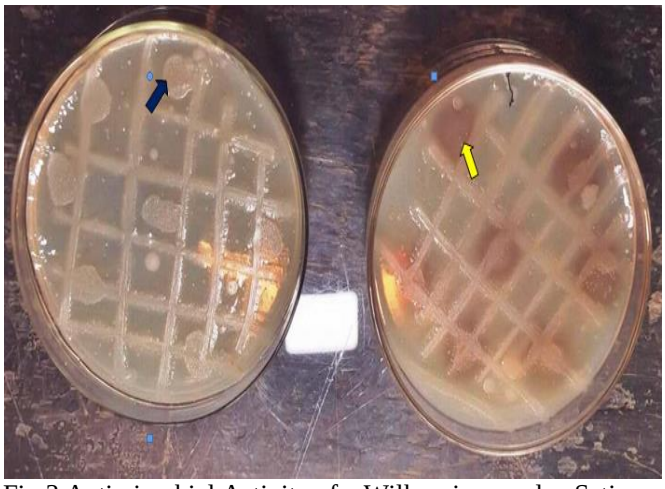

Fig 3 Antimicrobial Activity of a.Wilkensiana and a. Sativum as Separate Extracts.

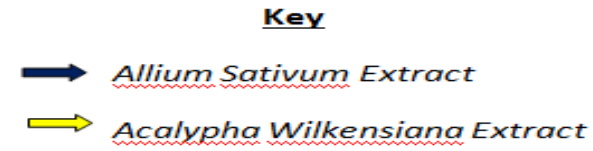

Fig 4 Milder Antimicrobial Activity Demonstrated by Individual Extracts of a Wilkensiana and a Sativum on E.Coli Colonies at 100mg/5ml Respectively

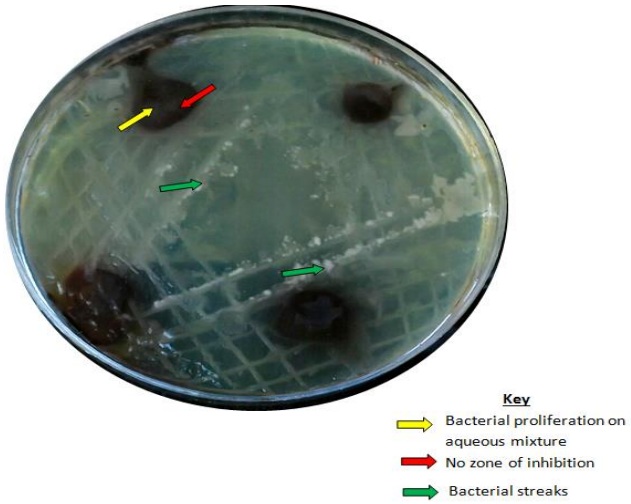

Fig 5 Antimicrobial Activity of Co-Administration of Acalypha Wilkensiana and Allium Sativum on E.Coli and Proteus Mirabilis Colonies at 100mg/5ml.

E. Antimicrobial Activity of Acalypha Wilkensiana and Allium Sativum extract mixture on E.Coli colonies at 100mg/5ml.

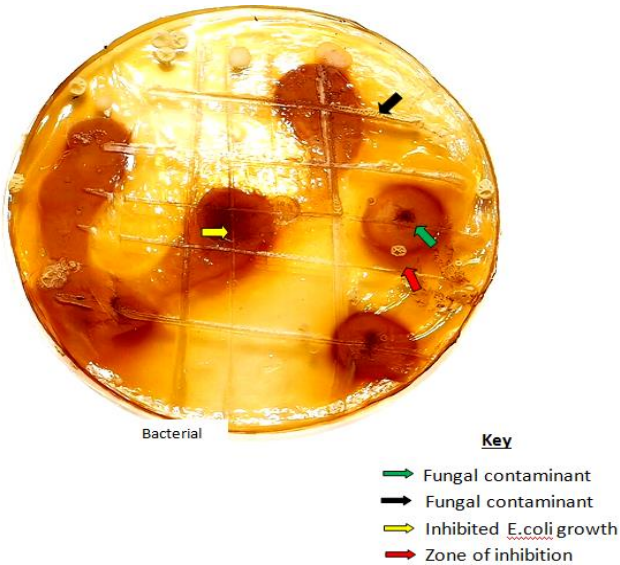

Fig 6 Absence of Antimicrobial Activity of extract mixture of A.wilkensian and A. sativum on mix colonies of E.coli and Proteus Mirabilis.

- Significant Antimicrobial Activity of A.wilkensiana and A. Sativum extracts mixture demonstrated on colonies of E.coli.

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V. CONCLUSION AND RECOMMENDATIONS

The sample mean and standard deviations of the parameters under study within the groups, exhibited characteristics of normal distributions, to inform the choice of the parametric, statistical tool, used (F –TEST). Thus, the demonstration of fulfilment of normality was ensured. This therefore means that samples were randomly selected; have equal variance and follow normal distribution (i.e possess SD that are less than one third their individual means).

The number of diseases reportedly managed by the use of *A. Wilkensiana* has made scientist seek the biochemical basis of its medicinal importance. One of such scientist are Gotep, et al (2016) who carried out an invitro antimicrobial screening using ethanol extracts of *A.wilkensiana* , and reported a marked sensitivity on micro-organism such as *S. Aureus* , *Yersinia enterocolitica*, *E.coli*, *S.typhi*, *P.aeroginosa* and *Klebsiella aeroginosa*.

Ogbuchi, et al(2014) studied the protective effect of *A.wilkensiana* on biomarkers of oxidative stress in liver homogenates , using 70% methanol extract which was administered intraperitoneally in a dose of 50mg/kg bw and 100mg/kg bw of the extract for a period of 14 days. A significant decrease ($p<0.05$) in malondialdehyde levels in the liver was registered; whereas, a significant increase($p<0.05$) in the activity of superoxide dismutase and catalyse in both 50mg/kg and 100mg/kg administered groups compared to control.

Cellini et al (1996) findings ,demonstrated that garlic extracts possess a broad spectrum of antimicrobial activity against certain gram positive and gram negative bacteria such as *E.coli, salmonella , staphylococci, streptococci, klebsiella, proteus, bacilli* and *clostridium* (Okoye 2006).

E.coli is a non –sporforming, facultative anaerobic gram negative Bacillus. It is an inhabitant of the intestine of warm blooded animals and is found in over 90% of humans. Although it represents less than 1% of intestinal microbiota, *E.coli* is the predominant aerobic organism in the gut. It is among the first bacterial species to colonize the intestine, establishing itself in the gut early after birth and remaining resident throughout the life of the host.

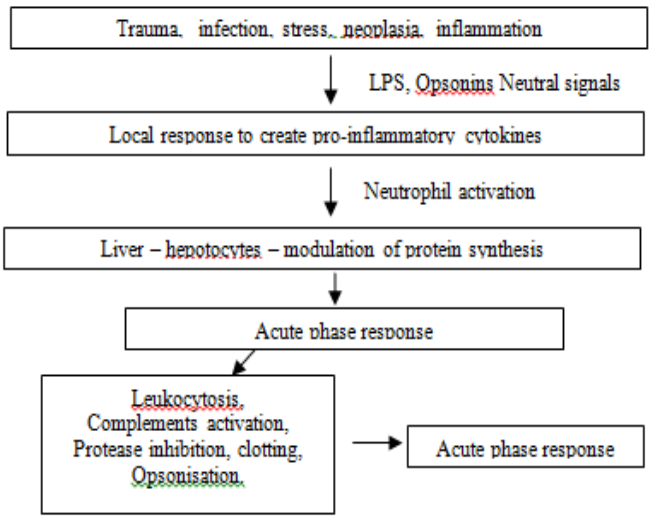
As a commensal, *E.coli* coexist harmoniously with its mammalian host, promoting normal intestinal homeostasis and preventing colonization by pathogens. However, some strains carry a combination of virulence genes that enable them to cause intestinal pathogenic *E.coli* (InPEC) and extra –intestinal pathogenic *E.coli* (ExPEC) infections (Martin 2015).

The isolation of *E.coli* in all the ten stool samples defines, completely, that *E.coli* is the culprit causing *kurga or makia –kia disease* of infants and neonates. Thus, it is safe to say that *kurga or makia –kia* is not a syndrome that depicts a clash between a weak or growing immune system and a normal flora, that has arrived too soon, but an actual disease. A recent update of a publication by Abdul Wasey and Philiip February (2019) which demonstrated the dangers in the *E.coli* strain 0157:H7 , first isolated in 1982 and its ability to secret *shiga* toxin which infects the alimentary tract and induces abdominal cramps , with hemorraghic diarrhoea, further buttress this point. This *E.coli* 0157:H7 strain has become a major worldwide food borne pathogen known to result in life – threatening conditions especially in children and infants, and ultimately leading to renal failure and a vast array of complications. Let me state here that more research needs to be done to determine the genetic characteristic of these isolated strains, so as to establish whether or not we are dealing with a common strain or an entirely new strain of *E.coli* that is not "friendly".

Again, one may ask, if *E.coli* is the culprit, why then the dermatological irregularities, listed as some of the symptoms of the disease? But then again, the answers to this question may be found in the phrase "Acute Phase Response". When the immune system is stimulated by an acute infection , trauma, inflammation, e.t.c, it leads to both biochemical and physiological changes called the " acute phase response" (APR),which is characterised by fevers ,skeletal muscle catabolism (as seen in patients with AIDS), increase synthesis of acute phase proteins (APP) like pre-albumin, prefibrinogen, C – reactive protein , serum amyloid A (SAA), e.t.c. Acute phase proteins (APP) are blood proteins primarily synthesized by hepatocytes as part of the acute phase response (APR). APR is part of the early defence or innate immune system, which is triggered by different stimuli including trauma, infection, stress, neoplasia, and inflammation. The APR may result in changes in more than 200 proteins grouped as either positive APP or negative APP.

In nearly all animal species, albumin represents the major negative APP, which, during the APR, decreases in blood concentration and may represent either selective loss of albumin due to renal or gastrointestinal changes or a decrease in hepatic synthesis. Positive APP are those that increase during the APR. They are further classified as major, moderate or minor, depending on the magnitude of increase during the APR.

A. Acute Phase Response



B. Conclusion

In conclusion, unless further scientific research proves otherwise , *kurga or makia-kia* is a bacterial disease of neonates and infants , caused by *E.coli*, regardless of its status as a normal flora . The single fact that it makes children ill, and mothers worried and uncertain is a characteristic of most diseases. Prior to this research , there exist no scientific basis to ascertain the existence of this disease and hence general refusal by health professionals to acknowledge its existence ,and therefore no known causative pathogen. These factors have led to the absence of effective therapy against the disease, .given rise to many undocumented mortalities among infants and neonates ,and is thus, gradually becoming a public health issue among local population.

The ability of the mixture of *A.wilkensiana* and allium sativum to reduce *E.coli* multiplication in the experimental animals while at the same time keeping important biomarkers within safe limits , confirms the claim by Gotep (2016) who carried out invitro antimicrobial screening using ethanol extracts.

C. Recommendation

I will greatly recommend further investigation to determine the exact chemical property of *A.wilkensiana* responsible for the observed antimicrobial activity. Similarly, genetic characterization of the isolated *E.coli* should be done and compared with the genome of existing strains archived in DNA banks, so as to bring to lime light, wether or not we are dealing with a new strain of the organism.

REFERENCES

[1]. Ageing, Australian Government Department of Health and. "Australian Paediatric Surveillance Unit annual report, 2010". www.health.gov.au. Retrieved 2017-12-16.

[2]. Anfimicrobial activity of crude Extracts of three modianal plats used in south - west Nigeria Folk medicine on some food Borne Bacterial pathogens. African Journal Traditional 314; 13-22.

[3]. Baucells, B.J.; Mercadal Hally, M.; Álvarez Sánchez, A.T.; Figueras Aloy, J. (2015). "Asociaciones de probióticos para la prevención de la enterocolitis necrosante y la reducción de la sepsis tardía y la mortalidad neonatal en recién nacidos pretérmino de menos de 1.500g: una revisión sistemática". Anales de Pediatría. 85 (5): 247–255. Polin, Richard (2014). Fetal and neonatal secrets. Philadelphia: Elsevier Saunders. ISBN 978-0-323-09139-8.

[4]. Bennett, John (2015). Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Philadelphia, PA: Elsevier/Saunders. ISBN 978-1-4557-4801-3; Access provided by the University of Pittsburgh.

[5]. Burkil, HM; (2004). The useful plants of West tropical Africa. Volume 6, 2nd Edition, Royal Atomic Gardenu, Kwe. 612.

[6]. Bennett, John (2015). Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Philadelphia, PA: Elsevier/Saunders. ISBN 978-1-4557-4801-3; Access provided by the University of Pittsburgh.

[7]. Cloherty, John (2012). Manual of neonatal care. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. ISBN 978-1-60831-777-6; Access provided by the University of Pittsburgh.

[8]. During, acute infection of a protein not normally present in the blood. J Exp. Med 73 73.173-182. Dutta AC (1998). Botany for Degree Students. Oxford University Press, India. 6th Edition, Page 587 - 634. "Epidemiological update: Outbreaks of Zika virus and complications potentially linked to the Zikavirus infection". European Centre for Disease Prevention and Control. Retrieved 18 January 2016.

[9]. Fanaroff, Avroy (2013). Klaus & Fanaroff's care of the high-risk neonate. Philadelphia, PA: Elsevier/Saunders. ISBN 978-1-4160-4001-9; Access provided by the University of Pittsburgh.

[10]. Florin, Todd (2011). Netter's pediatrics. Philadelphia, PA: Elsevier Saunders. ISBN 978-1-4377-1155-4. Gotep JG, Agada GOA, Gbise DS, Chollom 5 (2010) Antibacterial activity of ethanol extract of *Acalypha Wilkensiana* leaves growing in Jos, Plateau State, Nigeria. Mal J Microbiolog 6:69-74.

[11]. Gotep JG, Makoshi MS, O Ladipo OO, Forcados GE, Shu ML, Et al, (2016) safety evaluation of *Acalypha Wilkensiana* in Albino rats and BTK - 21 cell line, comparative clinical pathology 25: 1618 - 5641.

[12]. Gomaa, N.F., and Hashish, M.H., (2003). Journal Egypt Public Health association, 78 (5-6); 361372.

[13]. Groppo FC, Ramacciato JC, Simoes Rp, Florio Fm, Sartorattor A. Antimicrobial activity of garlic, tea tree oil, and chlorhexidine against oral microorganisms Int Dent J. 2002; 52: 433 - 437.

[14]. Gundiza, M, (1985). Photochemical servicing of Zimbabwean medicinal platas. The Central African Journal Med. (3); 326.

[15]. Hollier, Lisa M; Wendel, George D; Hollier, Lisa M (2008). "Third trimester antiviral prophylaxis for preventing maternal genital herpes simplex virus (HSV) recurrences and neonatal infection". Reviews (1): CD004946.

[16]. Health, Australian Government Department of. "Human Immunodeficiency virus (HIV)". www.health.gov.au. Retrieved 2017-12-16.

[17]. Hughes BG and Lawson DL. Antimicrobial effects of *Allium sativum* L. (Garlic). *Allium ampeloprasun* (elephant garlic), and *Allium cepa* (onion), garlic compounds and commercial garlic supplement products. Phytother Res. 1991; 5:154 - 158.

[18]. Abernathey JT , Avery OT. 1941. The occurrence. Alper CA 1974. Plasma protein measurements as a diagnostic aid.

[19]. Ikewuchi JC, Ikewudi CC (2010) Hypocholesterolaemic effect of aqueous extract o *Acalypha Wilkensiana* on rats fed egg yolk supplemented diet: Implications for cardiovascular risk management. Res J Sci Techn_ 2:78-81.
[20]. Isaacs, David (2014). Evidence-based neonatal infections. Chichester, West Sussex, UK: Wiley Blackwell. ISBN 978-0-470-65460-6; Access provided by the University of Pittsburgh. "Infectious diseases in pregnancy screening: programme overview; Detailed guidance". GOV.UK. 1 January 2015. Retrieved 2016-01-07.
[21]. Ikewuchi JC, Ikewudi CC (2010) Hypocholesterolaemic effect of aqueous extract o *Acalypha Wilkensiana* on rats fed egg yolk supplemented diet: Implications for cardiovascular risk management. Res J Sci Techn_ 2:78-81.
[22]. Ikewuchi JC, Ikewudi CC (2010) Hypocholesterolaemic effect of aqueous extract o *Acalypha Wilkensiana* on rats fed egg yolk supplemented diet: Implications for cardiovascular risk management. Res J Sci Techn_ 2:78-81.
[23]. Jadsada; Thinkhamrop, Bandit; Tolosa, Jorge E. (2014- 09-14). "Vaginal chlorhexidine during labour for preventing maternal and neonatal
[24]. Kumar, Ritu; Bronze, Michael Stuart (2015). "Pelvic Inflammatory Disease Empiric Therapy". Medscape. Retrieved 23 January 2019. Kunz C, Rodriguez-Palmero M, Koletzko B, Jensen R (June
[25]. Leveno, Kenneth (2013). Williams manual of pregnancy complications. New York: McGraw-Hill Medical. p. 507. ISBN 9780071765626.
[26]. Li, Shunming; Huang, Jingya; Chen, Zhiyao; Guo, Dan; Yao, Zhenjiang; Ye, Xiaohua (2017). "Antibiotic Prevention for Maternal Group B Streptococcal Colonization on Neonatal GBS-Related Adverse Outcomes: A Meta-Analysis". Frontiers in Microbiology. 8: 374. Ohlsson, A; Shah, VS (10 June 2014). "Intrapartum antibiotics "Listeria (Listeriosis)". Centers for Disease Control and Prevention. 22 October 2015. Retrieved 2015-12-23.
[27]. Leonardo Aguiar. "Ministério da Saúde confirma relação entre vírus Zika e microcefalia" [Ministry of Health confirms relationship between Zika virus and microcephaly].
[28]. Portal da Saúde – Ministério da Saúde. Archived from the original on 2016-01-29. Retrieved 2016-02-01.
[29]. MacDonald, Mhairi (2015). Avery's neonatology : pathophysiology and management of the newborn. Philadelphia: Wolters Kluwer. ISBN 978-1-4511-9268-1; Access provided by the University of Pittsburgh. for known maternal Group B streptococcal colonization". The Cochrane Database of Systematic Reviews (6): CD007467.
[30]. Martin, Richard (2015). Fanaroff and Martin's neonatal-perinatal medicine : diseases of the fetus and infant. Philadelphia, PA: Elsevier/Saunders. ISBN 978-1-4557-5617-9; Access provided by the University of Pittsburgh.
[31]. Mayhall, C (2012). Hospital epidemiology and infection control. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. ISBN 978-1-60831-300-6; Access provided by the University of Pittsburgh.
[32]. Mary T. Caserta (October 2015). "Overview of Neonatal Infections". Merck Sharp & Dohme Corporation. Retrieved January 16, 2015.
[33]. N Eng J med 291:287 - 290 Epubmed J. Angen **O,** Thomssen J, Lansen LE, Lansen J, Kokotovic, Hee Gaard PM, Enemark JM. 2009.
[34]. Neil K. Kaneshiro, David Zieve, Isla Ogilvie, A.D.A.M. Editorial team, eds. (December 4, 2013). "Neonate". U.S. National Library of Medicine. Retrieved January 16, 2016.
[35]. Okoye LA. In intro determination of bacterial effects of garlic *(Allium Sativum)* on *staphylococcus aureus* and *E. Coli*. NIJOSTEE. 2010; Vol. 3, No.1, ISSN: 03331. 9873. Akinemi, K. **O;** Oluwa, O. K. and Omonigbehin; E. **O** (2006).
[36]. Oliveira Melo, A. S.; Malinger, G.; Ximenes, R.; Szejnfeld, P. O.; Alves Sampaio, S.; Bispo de Filippis, A. M. (1 January 2016). "Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg?". Ultrasound in Obstetrics & Gynecology. 47 (1): 6–7.
[37]. Ohlsson, Arne; Shah, Vibhuti S; Stade, Brenda C; Ohlsson, Arne (2014). "Vaginal chlorhexidine during labour to prevent early-onset neonatal group B streptococcal infection". Reviews. 12 (12): CD003520. Lumbiganon, Pisake; Thinkhamrop,
[38]. Pryhuber, Gloria S. (2015). "Postnatal Infections and Immunology Affecting Chronic Lung Disease of Prematurity". Clinics in Perinatology. (4): 697–718. Access provided by the University of Pittsburgh.
[39]. Rubin Dasgrupta, Rajeridra S, Nancy and Rawat DS. Antibacterial effect of plants extracts on human microbial pathogero and microbial limit test. IJRPC, 2012; 2(4), ISSN: 2231. 2781.
[40]. Santosham, Mathuram; Chan, Grace J.; Lee, Anne CC; Baqui, Abdullah H.; Tan, Jingwen; Black, Robert E. (2013). "Risk of Early-Onset Neonatal Infection with Maternal Infection or Colonization: A Global Systematic Review and Meta-Analysis". PLoS Medicine. 10 (8): e1001502. Ann L Anderson-Berry, Linda L Bellig, Bryan L Ohning (December 31, 2015). "Neonatal Sepsis Clinical Presentation". WebMD LLC. Retrieved January 16, 2016. Siriwachirachai, Thitiporn; Sangkomkamhang, Ussanee S; Lumbiganon, Pisake; Laopaiboon, Malinee; Siriwachirachai, Thitiporn (2014). "Antibiotics for meconium-stained amniotic fluid in labour for preventing maternal and neonatal infections". Reviews (11): CD007772.

[41]. Sinha, Sunil (2012). Essential neonatal medicine. Chichester, West Sussex: John Wiley & Sons. ISBN 978-0-470-67040-8; Access provided by the University of Pittsburgh. Stoll, A, and seebeck, E., (2006). Chemical investigations on Allin, the specific principle of Garlic, Willey Online Library, Pg 11 - 35. "Tetanus and neonatal tetanus (NT)". WHO Western Pacific Region. Archived from the original on 2014-05-03.

[42]. Torgerson, Paul R; Mastroiacovo, Pierpaolo (2013). "The global burden of congenital toxoplasmosis: a systematic review" (PDF). Bulletin of the World Health Organization. 91 (7): 501–508. Ungerer, Regina LS; Lincetto, Ornella; McGuire, Van de Laar, Rafli; van der Ham, David P.; Oei, S. Guid; Willekes, Christine; Weiner, Carl P.; Mol, Ben W.J. (2009). "Accuracy of C-reactive protein determination in predicting chorioamnionitis and neonatal infection in pregnant women with premature rupture of membranes: A systematic review". European Journal of Obstetrics & Gynecology and Reproductive Biology. 147 (2): 124–129. "Grunting in Neonates - General Practice Notebook". www.gpnotebook.co.uk.

[43]. William; Saloojee, Haroon H; Gülmezoglu, A Metin; Ungerer, Regina LS (2004). "Prophylactic versus selective antibiotics for term newborn infants of mothers with risk factors for neonatal infection". Reviews (4): CD003957.

[44]. Zakaria Y. AI- Asta;. Effect of storage and temporaries of aqueous garlic extract. On the growth of certain pathogenic bacteria. Journal of Al Azhar University - Gaza 2003; Vol. 6,2 P. 11-20.

[45]. Zakher, Bernadette; Cantor MD, Amy G.; Daeges, Monica; Nelson MD, Heidi (December 16, 2014). "Review: Screening for Gonorrhea and Chlamydia: A Systematic Review for the U.S. Prevententive Services Task Force". Annals of Internal Medicine. 161 (12): 884–894. CiteSeerX 10.1.1.691.6232. Kenner, Carole (2014). Comprehensive neonatal nursing care (5th ed.). New York, NY: Springer Publishing Company, LLC. ISBN 978-0-8261-0975-0. Access provided by the University of Pittsburgh.